Long-term effects of severe acute malnutrition on growth, body composition, and function; a prospective cohort study in Malawi

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Declaration

I, Natasha Lelijveld, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed…… Date……


Malawi-Liverpool-Wellcome Trust
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I. Abstract

Background: Tackling severe acute malnutrition (SAM) is a global health priority. Whilst SAM-related mortality is well described, little is known about long-term effects on survivors. Given extensive evidence from developed countries that early nutritional insults have enduring detrimental effects, particularly heightened risk of non-communicable disease (NCD) in adulthood, it is plausible that SAM survivors could share some of these traits. Applying the DOHaD (Developmental Origins of Health and Disease) concept to a novel group, this study aimed to explore the long-term impacts of SAM on growth, body composition, and functional outcomes.

Methods and Findings: From August 2013 to February 2014 I followed-up the 352/1024 Malawian children who were still alive following SAM treatment in 2006-2007, comparing them to siblings and age/sex matched community controls. Compared to community controls, SAM-survivors had significantly lower height-for-age z-score (HAZ) (-0.4, CI -0.2 to -0.6) and limb length (-2.0cm, CI -1.0 to -3.0) although there was evidence of some catch-up growth. They also had smaller mid-upper arm, calf and hip circumferences and less lean mass. Functional deficits included: weaker hand-grip (-1.68kg, CI -0.9 to -2.4) and fewer minutes completed of an exercise test (-1.59 minutes, CI -1.0 to -2.5). There was no statistically significant impact on lung function, skinfold thickness, sitting height and head circumference.

Conclusions: These results show that there are long-term implications of an episode of SAM in the areas of growth, body composition, and physical function. Most notable are deficits in HAZ, lean mass, grip strength, and preservation of sitting height and head circumference at the expense of leg length, a pattern consistent with 'thrifty growth' which is associated with increased future cardiometabolic disease risk. Some catch-up of linear growth has occurred, suggesting that interventions post-"first 1000 days" could still be effective. Children who experienced SAM at an older age had greater detrimental effects, emphasising the need for early intervention and better prevention. Further follow-up is required to establish the full impact of puberty, and societal transition such as greater access to high fat/sugar diets. Intervention studies are also needed to determine whether earlier SAM treatment, improved post-SAM care such as nutrition supplementation and/or physical fitness interventions, could minimise or reverse some of these long-term effects.

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VII. List of Acronyms

A&E	Accident and Emergency Department
AMA	Arm Muscle Area
ARV	Antiretroviral
BAZ	BMI-for-age Z score
BIA	Bioelectrical Impedance Analysis
BIVA	BIA vector analysis
BMI	Body Mass Index
COPD	Chronic obstructive pulmonary disease
COT	Cotrimoxazole
CPET	Cardio-pulmonary Exercise Test
CVD	Cardiovascular disease
DHS	Demographic Health Survey
DLW	Doubly-labelled water
DOB	Date of Birth
DOHaD	Developmental origins of health and disease
ECG	Electrocardiogram
FEF_{25-75}	Forced expiratory flow during 25-75% FVC
FEV_1	Forced expiratory volume in 1 second
FFM	Fat-free mass
FM	Fat Mass
FVC	Forced vital capacity
GCP	Good Clinical Practice
GLI	Global Lung function Initiative
H2Z	Impedance Index
HAZ	Height-for-age z score
HIV	Human Immunodeficiency Virus
HR	Heart Rate
LBW	Low birth weight
LMI	Lean Mass Index
MAM	Moderate Acute Malnutrition
MDGs	Millennium Development Goals
MRI	Magnetic resonance imaging
MUAC	Mid-upper arm circumference
NCD	Non-communicable disease
OR	Odds ratio
OR	Odds Ratio
OTP	Outpatient Treatment Programme
PA	Phase Angle
PCA	Principle Component Analysis
PD	Post-discharge
QECH	Queen Elizabeth Central Hospital
R	Resistance (as measured by BIA)
RUTF	Ready-to-use Therapeutic Food
SAM	Severe Acute Malnutrition
SDGs	Sustainable Development Goals
SES	Socio-economic status
SRQ	Self-Reporting Questionnaire

TALC	Teaching Aids at Low Cost
TBW	Total body water
TEM	Technical error of measurement
WAZ	Weight-for-age z score
WHO	World Health Organisation
WHZ	Weight-for-height z score
Xc	Reactance (as measured by BIA)
Z	Impedance (as measured by BIA)

VIII. List of Publications and Presentations

2015-2016

- Oral presentation at Nutrition and Growth conference, Vienna March 2016. Long term effects of SAM on Growth and NCD risk signs
- Oral presentation at Royal Society of Tropical Medicine and Hygiene (RSTMH), London December 2015. Long term effects of acute malnutrition on growth, body composition and function in Malawian children
- Oral presentation at DOHaD Conference, Cape Town, November 2015. DOHaD and Severe Acute Malnutrition: A prospective cohort study of Malawian children
- Oral presentation at Wellcome Trust Annual Scientific Meeting (ASM), Malawi September 2015. Long term effects of acute malnutrition on growth, body composition and function.
- Poster presentation at European Respiratory Society (ERS) conference, Amsterdam September 2015. Long term effects of severe acute malnutrition on Lung Function in children http://erj.ersjournals.com/content/46/suppl_59/PA1257
- Poster presentation at European Respiratory Society (ERS) conference, Amsterdam September 2015. Kirkby J on behalf of other authors: Selecting a spirometry control group for Malawian children http://erj.ersjournals.com/content/46/suppl_59/PA1271
- Oral presentation during Plenary Session at RCPCH Conference, Birmingham 2015, Long-term Effects of Acute Malnutrition on Growth and Body Composition in Malawian children http://adc.bmj.com/content/100/Suppl_3/A2.2

2014

- Oral presentation at CommCare Advanced Topics Workshop, Blantyre, Malawi 2014. Using CommCare in academic study
- Oral presentation, ENN Technical Meeting 2014 (M. Kerac presented ChroSAM study on behalf of all authors) http://files.ennonline.net/attachments/2212/8.-Long-term-effects-SAM-on-stunting_FINAL.pdf
- Oral presentation at Wellcome trust 'annual scientific meeting' (ASM) (Award prize for Best Presentation in session), September 2014. Long-term effects of SAM in growth, body composition and function; a prospective cohort study in Malawi
- Oral presentation at European Respiratory Society (ERS) 2014, Munich (Kirby, J – on behalf of co-authors Kirby J, Lelijveld N, Kerac M, Stocks J.) Spirometry quality control grades in Malawian children http://erj.ersjournals.com/content/44/Suppl_58/215.short?rss=1
- Poster at IAEA Nutrition Conference, Vienna, May 2014. "Long term effect on Acute Malnutrition on Growth and Body Composition"
- Wasting and Stunting TIG meeting (technical interest group), London 2014. Long-term effects of SAM in growth, body composition and function; a prospective cohort study in Malawi

2013

- Oral Presentation at Research In Progress meeting, Malawi-Liverpool Wellcome Trust, Malawi 2013. Long-term effects of SAM in growth, body composition and function; a prospective cohort study in Malawi

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Chapter 1: Introduction

This chapter introduces severe acute malnutrition and some of the main concepts which justify my exploration of its potential long-term effects. I also introduce each of the four outcomes of interest for this PhD under individual subheading: growth, body composition, physical activity and lung function. The latter half of the chapter presents the scope of the study, the study hypotheses and the objectives.

1.1 Background

1.1.1 Severe Acute Malnutrition

Undernutrition is a major public health problem in developing countries and is recognised as an underlying factor in 45% of all child deaths[1]. Undernutrition can be chronic, acute, moderate or severe. Severe Acute Malnutrition (SAM) specifically affects more than 19 million children under 5 and causes more than 1 million child deaths each year[1, 2]. The diagnosis of malnutrition is based on measurement of body size (anthropometry) and clinical signs. SAM is currently defined as:

- having a low weight-for-height (<-3 z scores of the WHO 2006 international growth standard)
- or a low mid upper arm circumference (MUAC) (<115 mm)
- or presence of oedematous malnutrition (also known as Kwashiorkor)[3].

It is important to recognise that acute malnutrition is distinct from chronic malnutrition, which is defined as having a low height-for-age (<-3 z scores of the WHO standard) and is also known as stunting.

SAM, previously known as "protein-energy malnutrition" and with a variety of changing case definitions, has always been associated with high mortality. The majority of SAM-related research and programming has therefore traditionally focused on preventing SAM deaths[4]. However, in the last decade important strides have been made in the battle to curb child mortality; major advances in treating SAM [5] as well as many other child health interventions and social changes, have contributed to reductions in child mortality from the 10.8 million deaths per year estimated by the first Lancet Child Survival Series in 2003[6] to 6.3 million in 2013[7]. Increases in child survival has led policy and programmes across many fields of child health to focus more on preserving the quality of life and maximising child development, as well as reducing

mortality[2, 8]. This same shift in focus has contributed to the creation of an international malnutrition movement called Scaling Up Nutrition (SUN) which aims to prevent the long-term and irreversible impacts of malnutrition on intellectual, physical and social development, as well as increase GDP (Gross Domestic Product) in developing countries and reduce the burden on healthcare systems (http://scalingupnutrition.org/). These are all long-term and wider consequences of malnutrition, and much of the focus of the SUN campaign is on reducing stunting, known to have enduring detrimental effects.

The long-term effects of an episode of acute malnutrition are far less explored and therefore not a current focus for policy makers and programme implementers. For example, neither the Millennium Development Goals (MDGs) nor the new Sustainable Development Goals (SDGs) mention mitigation of the long-term effects of SAM. The SDGs do however mention targets for reducing wasting, stunting, and addressing premature mortality from non-communicable diseases, all areas neglected by the MDGs[9]. With SAM research focusing on mortality reduction and research for improving long-term outcomes of malnutrition focusing on stunting, research considering the long-term outcomes of SAM has fallen through the gap between these two priorities. As global child mortality falls, the long-term effects of SAM are becoming increasingly important. A recent major campaign called "Generation Nutrition" emphasises this need to look at "long term health effects" of SAM in point 8 of its10 point plan to end acute malnutrition[10]. For SAM programmes and policies to effectively achieve this, and in order to tackle all aspects of malnutrition, more evidence regarding the long-term effects of SAM is urgently needed.

1.1.1.1 SAM Pathophysiology

Understanding the pathophysiology of SAM is integral when considering the possible long-term consequences. SAM encompasses 2 related disorders: marasmus and kwashiorkor; it is also possible to have a combination of both known as marasmic-kwashiorkor. The term marasmus is derived from the Greek word *marasmos,* which means withering or wasting. The term kwashiorkor is taken from the Kwa language of Ghana and means "the sickness of the weaning" or "the deposed child", first used by Williams in 1933[11]. Marasmus is characterised by a loss of muscle mass assessed by anthropometry; weight-for-height and mid-upper arm circumference (MUAC) are the measures currently recommended by the WHO[12]. MUAC as a independent measure of wasting has only been widely accepted relatively recently, following an expert meeting in 2005[3]. However MUAC has been utilised as a valid measure of wasting for some time; for example the QUAC (Quaker Arm Circumference) stick which was a measure of MUAC for height, was first used in Nigeria in the 1960's[13]. Kwashiorkor is characterised by the presence of bilateral oedema starting at the feet and progressing up the body as severity increases.

It was previously thought that marasmus was the result of inadequate energy intake whereas kwashiorkor was the result of inadequate protein specifically[14]. However this has since been disproved as evidence has shown that children with kwashiorkor do not ingest any less protein than children with marasmus and oedema dissipates while children are being fed low protein diets[15, 16]. The exact difference between causes of marasmus vs kwashiorkor has yet to be elucidated. Prevalence of kwashiorkor is thought to be highest in central and southern Africa[17]; it is particularly high in Malawi where the majority of SAM cases present with oedema[18].

In both marasmus and kwashiorkor normal physiology is disturbed by a process called 'reductive adaptation'. This process involves catabolizing tissue reserves of carbohydrate, fat, and protein , and reducing activity of all physiological and metabolic body systems in order to adapt to a lack of nutrients[19]. Physical activity and growth are reduced and the basal metabolic rate is slowed. This process is initially beneficial, allowing maintenance of homeostasis though reduced energy demands however the resultant metabolic changes become progressively more dangerous[20]. The number and function of sodium-potassium (Na-K) pumps in cell membranes is reduced resulting in sodium leakage into cells and loss of potassium out of the cells and into the urine. The function of all the major organs is also impaired: the kidneys are less able to excrete the extra sodium; cardiac muscle is atrophied and hypokalaemia also contributes to reduced cardiac function. Glucose stores in the liver are depleted and gluconeogenesis impaired. Nutrient digestion and absorption is impaired by villous atrophy, minimized production of acid and enzymes, and decreased intestinal muscle function, which can compound the lack of nutrients available[19]. The immune and inflammatory systems also become impaired, resulting in vulnerability to infections; pneumonia, TB, enteric infections and malaria are common complications in SAM patients[20, 21].

Understanding these changes in physiological function is very important in the management of SAM treatment; therapeutic decisions that are life-saving in a well-nourished child can be potentially fatal in a child with SAM. Due to the atrophied cardiac muscle and increase in body sodium, children with SAM are very sensitive to increases in circulatory volume. Their metabolism also cannot cope with high levels of protein whilst in a state of reductive adaptation hence feeding treatment must start with a low protein, low energy, and high micronutrient food. Children are not expected to gain weight until after metabolic equilibrium has been reobtained; this "stabilisation phase" is then followed by "recovery phase" where patients can be provided with high quantities of energy, protein and the right balance of macro and micronutrients to promote tissue growth and weight gain[20]. The WHO recommends "ten steps" within these phases in order to best manage children with SAM; steps one to six focus on managing the

changes in physiological function seem in SAM children: hypoglycaemia, hypothermia, dehydration, electrolyte imbalance, infection and micronutrient deficiencies[22]. The ‘reductive adaptation”, the concurrent infections and the treatment process could all have implications for long-term outcomes following SAM, particularly during a time of fast development such as the first few years of life.

1.1.1.2 Causes of SAM

Understanding some of the causes of SAM may also have relevance to the possible resultant long-term effects. The underlying causes can be complex, involving not only nutrition but other health factors, agriculture, seasonality, maternal health, culture and socioeconomic factors. Malnutrition, chronic and acute, may result from a combination of inadequate nutrient intake, increased losses (diarrhoeal episodes, vomiting) and increased energy expenditure (usually due to infections). Acute malnutrition often presents around the time of weaning (hence kwashiorkor translates as "weaning sickness") because it is a critical period when breast milk no longer provides adequate calories for growth but weaning foods may be nutritionally incomplete and also a source of infection[19].

We now know that nutrition and infection have a cyclical relationship[23], both playing an important role in SAM. It is often difficult to determine the exact sequence of events in any one child as malnutrition and infection coexist with one increasing susceptibility to the other. Factors therefore that contribute to either poor diet and/or infection can be underlying causes of SAM. These factors can range from immediate causes such as diarrheal infection to high-level "basic" causes such as national resources and international economic structures, as described in the UNICEF conceptual framework (**Figure 1**).

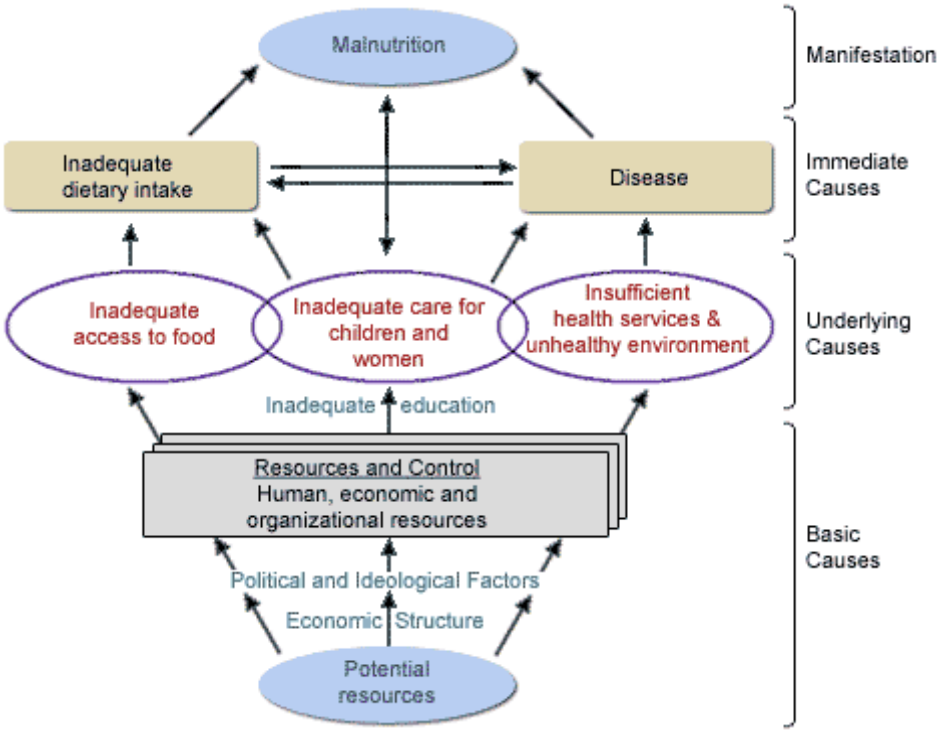


Figure 1: UNICEF conceptual framework for causes of undernutrition[24]

Besides the increased energy expenditure, anorexia and direct losses that accompany enteric infection, there is growing appreciations that these infections impair intestinal absorption which may be contributing to a substantial portion of global malnutrition[25]. The lumen of the gastrointestinal tract is lined by a single layer of cells: the epithelium. Malnutrition and diarrhoea result in damage to the lumen epithelial cells which can hamper absorption and makes them susceptible to infection; this can then lead to further malnutrition and enteric infections. This is thought to be another element to the infection-malnutrition cycle; it could also be an important consideration for SAM survivors who may have even poorer nutrient absorption than other children as a result. A recent high-profile study found evidence that gut microbiome of SAM survivors remain suboptimal or "immature", even after full weight recovery[26]. The researchers suggested that these immature gut microbiomes may result in long-term implications such as stunted growth, cognitive delays, and weakened immune system.

1.1.2 Early Life Nutrition and Long-term Health

"*The devil has put a penalty on all things we enjoy in life. Either we suffer in health or we suffer in soul or we get fat*." - Albert Einstein

As our understanding of SAM physiology and intestinal infections has increased, SAM treatment has improved resulting in a striking reduction of mortality among children with SAM[27]. Whilst the focus on nutrition research in developing countries has so far focused on how best to meet nutritional needs and bringing down mortality rates, nutrition research in developed countries has shifted to understanding the effects of nutrition on long term health.

There is now a large evidence base linking early life nutrition and adult health, particularly non-communicable diseases (NCDs) in adult life. Some of the earliest evidence linking nutrition and long-term health came from animal models, when in 1933 it was found that restricting diets of rats in early life resulted in increased life span[28]. The concept began to attract a lot more attention following the 1995 publication of a key theory based on human evidence linking low birth weight (LBW) and coronary heart disease in adulthood: Foetal Origins of Adult Disease hypothesis, also known as The Thrifty Phenotype hypothesis [29, 30]. LBW is most commonly defined as an infant weighing less than 2500g at birth[31]. The theory proposes that environmental factors in utero such as malnutrition can result in metabolic adaptations which may favour immediate survival but that program future propensity to adult diseases. Strong evidence now links early life nutrition to risk of diabetes, obesity, hypertension, dyslipidemia, cardiovascular disease (CVD), chronic obstructive pulmonary disease (COPD) and asthma[32, 33]. The majority of this evidence focuses on prenatal nutrition, generally using LBW as a measure of prenatal undernutrition. There have however been a few acute historical famines

which have enabled researchers to link prenatal undernutrition with later health outcomes without the need to define malnutrition as LBW.

1.1.2.1 Using "Natural experiments" to Link Early Nutrition to Later Health

Studying historic famines in developed countries allows for retrospective cohorts diminishing the need to follow-up a group of individuals over many decades. These studies have tended to take place in developed countries where individuals are otherwise healthy and subsequently well-nourished, reducing the number of confounding factors. One key example of a "natural experiment" is the Dutch famine and its resultant studies. These studies used a cohort of adults known to be in utero during the time of the Dutch famine. This famine was specific in both timing and location as it resulted from a food shortage due to a blockade by Allied forces during World War II. Studies of this cohort have linked in-utero nutrition to impaired glucose tolerance, hypercholesterolaemia, raised blood pressure and obesity. These studies even allowed for analysis of the nutritional deficiency and resultant adult outcome to be linked to specific gestational periods[34].

Another key series of famine studies comes from those exposed to the siege of Leningrad by German troops, also during World War II. This siege lasted from September 1941 to January 1944 resulting in an acute food shortage and severe malnutrition. It is estimated that the average daily ration per person during the most severe period of the siege was around 300 kilocalories and contained virtually no protein[35]. However, in contrast to results from the Dutch Famine, survivors of the siege of Leningrad exposed to famine either in utero or infancy did not have significantly different insulin concentration, fasting glucose, blood pressure, or concentration of lipids or coagulation factors than unexposed controls[35]. There was however evidence that endothelial damage was raised in the subjects exposed to intrauterine malnutrition. It has been suggested that these survivors were not affected in the same way as survivors of the Dutch Famine because they remained relatively poor and undernourished even after the siege had finished, whereas the Dutch Famine survivors quickly reverted to a higher socioeconomic state and were therefore exposed to a larger contrast in nutrition conditions[36].

A third example of famine exposure and later health comes from the 1959–1961 Chinese Famine, the largest in human history. A number of interrelated factors including drought, industrialisation and the social structure of the "Great Leap Forward" resulting in decreased incentives for food production, and a delayed response to the food shortage by the authorities all contributed to the famine which caused millions of deaths[36]. Studies using extensive government health records have found that foetal exposure to the famine was associated with increased risks of overweight, schizophrenia and hyperglycaemia in adult life [36-38]. As well as foetal exposure, studies have considered the long-term outcomes of those exposed to the famine during early and late

childhood; results suggest that postnatal exposure during the first 3 years of life reduces adult height and increases adult BMI, risk of hypertension and possibly risk of hyperglycaemia[36, 38]. Evidence for the long-term impacts of postnatal nutritional insults may be most relevant for SAM survivors as the majority of SAM cases are between the ages of 18 months and 3 years.

One final example, also very relevant as it is from an African context, is a study of those exposed to the famine in Biafra during the Nigerian civil war (1967–1970)[39]. Forty years later, this study found that foetal-infant exposure to this famine was associated with elevated BP, increased waist circumference, and increased risk of systolic hypertension, impaired glucose tolerance, and overweight, as compared to people born after the famine. Although the study did not have access birth weight data and was unable to separate the effects of foetal and infant famine, the results are highly suggestive of potential adverse consequences in SAM survivors across the continent.

1.1.2.2 Postnatal Nutrition and Long-term Health

Besides evidence from the Chinese Famine, there are some other examples of studies showing the long-term effects of postnatal nutrition. These include evidence of the protective benefits of breastfeeding and studies reporting the negative consequences of rapid weight gain in the first few months of life[40, 41]. In fact, some now argue that much of the detrimental effects of LBW could actually be attributed to the rapid postnatal catch up growth experienced by LBW infants[42]. As this evidence has widened the window of plasticity in which nutritional insults can occur, the theory previously known as the "foetal origins of adult disease" hypothesis is now commonly known and accepted as the Developmental Origins of Health and Disease (DOHaD) theory[43]. **Table 1** summarises the literature on human studies linking early nutritional insults to later health, highlighting not only the plethora of outcomes but the complexity of when, how, and what. The evidence linking rapid weight gain to detrimental adult health may also be relevant for SAM survivors who experience rapid weight gain during treatment. Rapid weight gain is currently used as a measure of SAM treatment performance but may not be consistent with optimal longer-term health outcomes. There is need for more evidence during SAM treatment regarding the type of weight gain undertaken at this time and resultant body composition changes. Although this study will not be able to decipher whether the undernutrition or the subsequent rapid weight gain is the cause of any adverse long-term outcomes, this is something which can be considered by future studies and could have great implications for how SAM is treated in the future.

Trait Influenced (Human studies only)	Nature and Timing of nutritional insult/ nutrition choice	Reference
Cardiovascular risk (inc. Atherosclerosis)	Prenatal growth retardation Rapid infant catch-up Formula-feeding	Barker 1995[29]; Huxley 2007[44]; Forsen 1999[45]; Leon 1998[46]; Singhal 2004[47] & 2004[41]
Hypertension	Prenatal growth retardation Rapid infant catch-up Formula-feeding Early postnatal diet quality	Martin 2005[48]; Lawlor 2005[49]; Brion 2008[50]; Ekelund 2007[51]; Gennser 1988[52] Huang 2010 [38]
Glucose intolerance/insulin sensitivity/ diabetes	Prenatal growth retardation Rapid infant catch-up Formula-feeding	Owen 2006[53]; Hales 1991[54]; Ravelli 1998[55]; Soto 2003[56]
Skeletal muscle development	Prenatal micronutrient deficiency	Iannotti 2008[57]
Cognitive Function	Early postnatal diet quality SAM	Smithers 2012[58]; Golley 2013[59]; Grantham-McGregor 1994[60]
Obesity	Prenatal growth retardation Rapid infant catch-up Early postnatal diet quality	Ravelli 1975[61] & 1999[62]; Lucas 1999[42]; Singhal 2004[41]; Harder 2005[63]; Schack-Nielsen 2010[64]; Owen 2005[65]
Dyslipidaemia / HDL Cholesterol	Prenatal growth retardation Rapid infant catch-up Formula-feeding	Owen 2002[66]; Wells 2007[67]; Fagerberg 2004[68]; Singhal 2004[41]
Endothelial dysfunction	Rapid infant catch-up	Singhal 2004[47]
BMI / Abdominal adiposity	Prenatal growth retardation Rapid infant catch-up Early Postnatal diet quality	Fagerberg 2004[68]; Ibanez 2008[69]; Kensara 2005[70]; Singhal 2010[71]; Meyerkort 2012[72]
COPD / Asthma	Prenatal growth retardation	Barker 1991[73]; Svanes 2010[74]
Schizophrenia	Prenatal famine exposure	St Clair 2005[37]; Hoek 1998[75]

Table 1: Summary of the evidence and references from human studies linking early nutritional insults to later health, including a list of long-term outcomes as well as the nature and timing of the insult/ choice

1.1.2.3 Mechanisms and "The window of Plasticity"

The mechanisms underlying DOHaD are currently not clear. Current theories include premature telomere shortening, disruption of organ function which affects hormone secretions, increases in the number or size of fat cells, alterations in adipose tissue function; and/or disturbances in regulation of appetite caused by central nervous system dysfunction [76]. Another theory is the "capacity-load" model which pins the mechanism on organ size and lean mass levels; if the growth of organs is hampered during early life and an individual gains weight in adulthood thereby increasing their metabolic load, their capacity is insufficient making them susceptible to NCDs[77]. The mechanisms have been difficult to define due to the long gap between exposure and outcome, with timing, the multitude of confounders, and selection bias making conclusions problematic [78]. Identification of the mechanism(s) controlling this link between early nutrition and adult health will likely aid in further defining the window of plasticity, explain how both low and high birthweight are associated with NCDs and finally help in designing interventions. We currently know that the "window of plasticity" starts in utero and continues into the first few months of life, and potentially beyond. Phenotypic plasticity is defined as the ability of a single genotype to produce more than one alternative form of morphology, physiological state, and/or

behaviour in response to environmental conditions[79]. The window for developmental phenotypic plasticity is finite; plant and animal studies have shown that the window for phenotypic adaption to the environment varies greatly across species and can either be very narrow or very wide[80, 81]. How large the window is in humans is not known; as humans are born at a relatively advanced state in development, it could be that phenotypic plasticity largely stops around early infancy and that the adaptions seen in LBW babies are not relevant to SAM children. However, we do know that a large portion of growth and development happens after birth; hence it could be that infants suffering from SAM are subject to phenotypic adaptation. This study aims to add evidence to DOHaD in a completely new patient group, as well as help to define the extent of the "window of plasticity" for DOHaD in human children.

1.1.2.4 DOHaD in Developing Countries

The study will also contribute to the currently limited evidence for DOHaD in developing countries, as the majority of studies in this area have so far been conducted in developed nations. The implications of DOHaD for developing countries are just beginning to be more widely discussed[82]. Nutrition research and interventions in developing countries have largely focused on preventing and treating undernutrition, which remains an important problem, however it is now becoming clear that NCDs are also of top priority for global public health. In 2008 80% of the 36 million deaths caused by CVD, diabetes, cancer and COPD occurred in low and middle-income countries[83]. The effects of early life nutrition in developing countries with reference to NCDs in adulthood is therefore of high importance.

Besides foetal and infant nutrition, there has been significant research in developing countries into the long-term effects of chronic undernutrition in childhood (stunting) on intellectual ability, economic productivity, reproductive health and some chronic diseases. However, again, the biological mechanisms behind these links are not clear and the complicated web of co-determinants is difficult to unpick. Additionally, there is evidence that stunting and foetal nutrition are intertwined[84], so is it stunting which results in poor adult health and productivity? Or is this on the causal pathway of low birth weight and/or maternal undernutrition? This picture becomes even more complicated when considering the links between SAM and stunting. The relationship between weight-for-height and height-for-age are still not fully understood and the evidence so far has been inconsistent depending on the type of data being considered (i.e. population or individual level; cross-sectional or longitudinal)[85].

1.1.3 Long-term Effects of SAM

Few studies have explored the long-term effects of SAM specifically. One recent study conducted a literature review on the long-term outcomes of SAM patients and found eight existing papers[86]. Most of these studies followed-up children for between 6 and 18 months after discharge, including a paper which describes the one-year follow-up of the cohort being used in this study[87]. This initial follow-up of 1024 children admitted to "Moyo" Nutrition ward in Blantyre, Malawi in 2006 and 2007[88] found that 23% (238) died during the inpatient phase of treatment; a further 8% (84) died in the first 90 days following original admission; and at 1 year post-discharge only 45% were reliably reported to still be alive. The "FuSAM" 1 year follow-up also found that, using the NCHS 1978 growth reference, mean weight-for-age z-score (WAZ) was significantly lower for cases than sibling controls, as was mean height-for-age z-score (HAZ).

Very few studies have gone on to follow up ex-SAM children for longer than 18 months, particularly using the current definitions of SAM and since the onset of the HIV pandemic [89, 90]. One relatively recent study from India in 1999 followed up SAM survivors ranging from 1 and 7 years post-discharge and compared their growth to that of their siblings. Interestingly, they found SAM-survivors to be in better health than their siblings which could be explained by "survivor bias"[91]. Survivor bias will also be an important consideration for this study as we already know that 42% (427/1024) of the case children had died by 1 year post-discharge. HIV will also be a pertinent consideration in this study as 49% (445/904) of the original case group were known to be HIV positive. The HIV positive group may also be particularly susceptible to survivor bias as of those children known to have died by 1 year post-discharge, 70% (298/427) were known to be HIV positive.

The focus of malnutrition interventions and research in developing countries has very much been focused on children less than 2 years old, and this has been galvanised in the past few years by the "thousand days" concept which states that the optimal "window of opportunity" for preventing long-term consequences of malnutrition is in the first 1000 days of life (conception to 2 years) (www.thousanddays.org)[92]. This catchy concept has gained considerable political backing and investment. Some are concerned that the success of this campaign may have resulted in the view that interventions outside of this window are unlikely to have any effect[93]. There is some evidence that stunting can be partially or fully reversed during adolescence, and there is an effort being made to dub this the "second window of opportunity" lest it be forgotten[93, 94]. With regard to SAM, first the long-term effects need to be established in order to determine where the windows of opportunity for prevention and reversal might lie.

Based on strong evidence, largely from developed countries, that nutrition in prenatal and early post-natal life can affect long-term health, particularly NCDs, it is conceivable that SAM may

have adverse long-term effects. NCDs are increasingly becoming a burden in developing countries, including Malawi, and it is thought that traditional risk factors, such as smoking, obesity, high salt intake, sedentary lifestyle and pollution, may not fully explain the rapid increase in the NCD epidemic and the early onset of disease in sub-Saharan Africa. Other unique factors specific to the region, such as high levels of child undernutrition, are now also thought to be important[95]. However there is currently no research to support this. This study is an exploratory start at filling this research gap.

1.1.4 Study Outcomes: Growth, Body Composition, Physical Function and Lung Function

1.1.4.1 Scope of the study

Based on current evidence about DOHaD, the long term effects of SAM are likely to be changes in growth, body composition, and organ function and metabolism, which may contribute to an increased risk of NCDs in adulthood. As this is an exploratory study, it aimed to look at a large variety of potential adverse outcomes including some of the immediate changes as well as early risk signs for NCDs. However, every study must have its limits and my PhD also had to have limits. This PhD therefore focuses on the most fundamental long-term outcomes, namely growth and body composition, which enable a detailed description of the child's current phenotype. I also wanted to take these outcomes one step further to consider if these were having an effect on the child's everyday function, namely physical activity, muscle strength, physical capacity, and lung function. Both the phenotypic and functional outcomes all have potential implications for NCDs in adulthood, which was another important consideration.

Beyond my PhD, the larger study that I was working on also considered various aspects of the other major body systems: lipid profile, endocrine function, cognitive function, and cardiovascular function. Each of these areas could be large studies in themselves; hence I decided to leave these out of my PhD as it would not be possible to adequately discuss them all. This study focused on whether there are long-term effects of SAM and what they might be, it did not intend to establish which mechanisms are responsible for these effects; that is the role of future research.

The rest of this section explores some of the evidence specific to the long-term outcomes being assessed in this study (growth, body composition, physical function and lung function) and begins to make hypotheses based on this evidence, starting with growth.

1.1.4.2 Long-term Effects of Nutrition on Growth Outcomes

Growth is an important determinant of both short- and long-term health and quality of life[84, 96]. In 2006, Kerac et al. conducted a 1 year follow-up of SAM survivors discharged from the Nutrition Rehabilitation Unit (NRU) in Blantyre, Malawi, and found that not only had a large proportion (10%) of those discharged as cured since died, but the height-for-age of survivors remained very low. Mean HAZ of survivors was -3.37 (or -2.97 based on NCHS growth reference) compared to 2010 DHS mean HAZ -1.8 in Malawian children <5 [97, 98]. Stunting has known long-term adverse effects including reduced earning capacity, lower cognitive function and poor outcomes during child birth, as well as adverse inter-generational effects[84].The relationship between wasting and stunting is currently poorly understood; SAM is usually considered to be a short-term response to inadequate food or a result of acute infectious disease, whereas stunting is considered to be a gradual response to chronic poor diet or repeated infections[85, 99]. There is a large variety of conflicting results from cohort and cross-sectional studies exploring if and how much SAM contributes to stunting [100-105]. Findings from the 1 year follow-up of this cohort suggested that SAM survivors are at greater risk of stunting in the short-term; I will explore the prevalence of stunting in SAM survivors 7 years post-discharge.

Although stunting has known adverse outcomes, whether stunted children can reverse this affliction has also been studied with mixed results[103, 104]. Catch-up growth in children who remain in the same environment with minimal change in diet and prevalence of infections is thought to be difficult; infections can result in wasting as well as loss of linear growth, and insufficient food means there is little opportunity for catch-up [84, 94]. Two well-known cohort studies have presented conflicting evidence with regard to catch-up growth in stunted children; Adair et al., found stunted Filipino children were able to catch-up a significant amount of their HAZ by adolescence without intervention, whereas Martorell et al,. found that a Guatemalan cohort remained as stunted by late adolescence as they were in early childhood [103, 106]. More specific to this cohort, a study in India followed-up two groups of SAM survivors: those discharged 1-3 years ago and those discharged 5-7 years ago, and found large improvements in weight-for-age in both groups but only found improvements in height-for-age in those children discharged 5 -7 years ago[91]. This could therefore be true for our cohort, who exhibited no catch-up in HAZ by 1 year post-discharge but could have had some catch-up growth by 7 years post-discharge. Remarkably, the India follow-up study also found that weigh-for-height was actually lower in the sibling controls than SAM survivors; they hypothesised that this is due to survivor bias. Kerac at al., did not find this pattern at the 1 year follow-up[97], but it is something which needs to be kept in mind when interpreting the results of this follow-up. It is also worth mentioning the value of control groups in assessing both stunting and catch-up growth. Deficits or improvements in HAZ compared to a global reference could merely reflect changes in isolated

household or community environments whereas comparing HAZ and change in HAZ to local control groups allows for these factors and makes a stronger scientific argument for the specific influences of SAM.

Another consideration is the age at which catch-up growth is likely to occur. The majority of our cohort were admitted with SAM when they were between 1 and 3 years old, and they are now between the ages of 8 and 11 years. Some cohort studies have concluded that although catch-up growth does not occur in childhood, it can occur in adolescence [93, 99, 107]. One study found that further growth faltering can occur throughout childhood [108]. Due to the large mixture of evidence, I conservatively hypothesise that SAM survivors will remain significantly more stunted than their sibling and community controls, and that their growth velocity between 1 year and 7 years post-discharge will be similar to their sibling controls, not accelerated. In order for this group to catch-up to their community or family "norm" they would need to grow on a steeper growth trajectory. There is some evidence to suggest that this catch–up growth is more likely to occur in adolescence rather than in childhood. This is biologically plausible as most tissues, besides neuronal tissues, still have considerable growth and development to undergo between 24 months and adulthood, particularly musculoskeletal tissue which, as a proportion of total growth, slows from ages 4 to 12 years, and then increases rapidly during adolescence (**Figure 2**)[93, 109].

Besides linear growth, **Figure 2** and other existing data suggest that SAM survivors will not have significantly different head circumference to controls as the majority of head growth has already occurred by the age of 2 years, before the SAM insult occurred in many. Additionally, based on Hales and Barker's original "Thrifty Phenotype" hypothesis and the concept of "grow now, pay later", core organ function and vital growth is preserved in times of limited resources at the expense of some less vital areas of growth and development. This suggests brain growth and head circumference are likely to be preserved in all but the most extreme cases of malnutrition, as evidence from LBW infants and undernourished children has shown [30, 40, 110, 111].

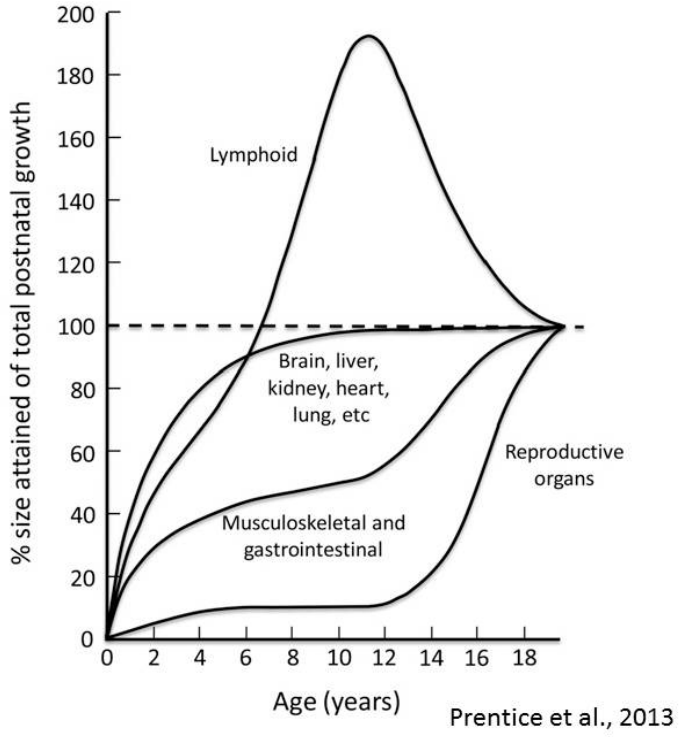


Figure 2: Differential timing of growth of major body systems from birth to 18 years in humans. Source: Prentice et al.[93]

An area of "less vital" growth which may be compromised during a period of limited resources such as SAM is limb length. Human stature is made up of approximately 50% legs, and 50% trunk and head but there is existing evidence to suggest that early life insults may reduce leg length resulting in disproportioned bodies[112, 113]. There are several theories about exactly which tissues in the legs are affected and why. One theory states that the further from the heart the tissue, the less of a priority it is for growth[114, 115]. Another theory states that those tissues which grow the fastest are the most effected during an episode of illness or malnutrition and legs, especially the tibia, are growing the fastest in relation to other body segments between birth and 7 years [112]. Leitch (1951) was the first to propose that a ratio of leg length to total stature could be a good indicator of early life nutrition [116]. Leg length has since been associated with length of breastfeeding, iodine deficiency, socioeconomic status and maternal smoking [117-122]. Having disproportionately shorter legs has also been found to have considerable consequences on adult health, such as higher risk of overweight, coronary heart disease, high blood pressure and diabetes [121, 123, 124]. Sitting height to height ratio has been found to be associated with overweight, coronary heart disease and diabetes in adulthood[121, 123]. In a developing county context, one study found that chronic undernutrition resulting in stunting did not result in disproportioned sitting height ratio[125]. This study therefore hypothesises that SAM survivors will have relatively shorter legs than control children due to the acute insult suffered by SAM survivors, opposed to the chronic undernutrition likely suffered by some controls.

1.1.4.3 Long-term Effects of Nutrition on Body Composition Outcomes

Besides impacts of long-term growth, an insult such as malnutrition during a critical time in development can also result in altered body composition in later life[67]. The "Thrifty Phenotype" hypothesis (one theory under the relatively new "DOHaD" umbrella) postulates that if resources are limited during early development this induces an adaptive response which is protective at that time and increases chances of immediate survival. A now dated theory was that in most cases, after the insult had passed, survivors were exposed to adequate or excess resources, resulting in rapid catch-up growth and a *mismatch* of phenotypic adaption and actual environment[126, 127]. Although we now know that those exposed to poor nutrition in early life do worse in all adult environments, including famine[128], it may still hold true that the phenotypic adaptations which were protective in early life become a disadvantage later on. This may explain the strong evidence linking rapid catch-up growth following prenatal malnutrition with greater chances of obesity in both childhood and adulthood, as well as altered ratios of lean and fat mass[67, 129] however the exact mechanism for this is not currently known. Barker suggested that the adaptive phenotype during undernutrition favours fat storage, particularly on the core body where resources are needed for vital organ function and development, and this phenotype continues post-malnutrition[29].

Body composition is a measure of the amount and distribution of fat and fat-free mass in the body, as well as the body's general size and shape. Not only the amount of fat, but the positioning of fat is of great importance to health, particularly NCDs in adulthood. Central fat on the waist and core body is detrimental to health, whereas peripheral fat on the hips and limbs is protective against some health problems [130-132]. Storage of fat on the core body can lead to an increase in fatty acids being released into the blood stream and therefore increased blood pressure, more insulin resistance and higher serum cholesterol. This also leads to an increased synthesis of triglycerides and very-low-density lipoprotein (VLDL) secretion by the liver, which is associated with CVD risk[133, 134]. The adverse effects of increased core fat storage may even be compounded for those with early undernutrition as organ and body size are likely to be smaller and therefore less able to cope with increased metabolic load. Metabolic load can be defined as the amount of tissue making demands on organs as well as things that challenge fuel homeostasis in other ways such as sedentary behaviour and smoking[67]. In other words, those with early nutritional insults are more likely to accumulate core fat due to phenotypic adaptions and are also more likely to have reduced "metabolic capacity" due to their smaller/damaged organs. This however does not explain the U-shaped relationship seen with birth weight and obesity where both low and high birth weights are associated with later obesity and subsequent NCDs[135].

So far, evidence has been found linking an increased proportion of core body fat and related NCDs to both prenatal and postnatal nutritional insults; namely LBW, preterm births, rapid

weight gain in infancy, and formula milk feeding. This study aims to assess whether SAM in childhood can also be added to the list of early nutritional insults which affect later body composition. I hypothesise that SAM survivors will have less peripheral fat and more central adiposity than community and sibling controls. I do not expect cases or controls to have large amounts of fat as the majority are known to come from very low socioeconomic environments, however evidence shows that a person does not need to have a lot of fat to suffer the adverse consequences of altered body composition[136]. India has the highest incidence of diabetes in the world, and Indian diabetic patients have been described as "*thin but fat*", meaning they are thin because they have low BMI, short height for age and low levels of lean mass, but fat because they have higher waist hip ratios, higher central subcutaneous fat levels and higher body fat percentages than non-diabetic Indians[136]. This pattern of "*thin but fat*" seems like a realistic expectation for the SAM survivors in this study. I also hypothesise that not only will SAM survivors have less peripheral fat than controls, they will have less peripheral lean mass than controls; both peripheral fat and peripheral lean mass are thought to be protective against NCDs in adulthood[67, 131]. Iannotti et al [57] for example found that calf circumference was significantly larger in infants whose mothers were treated with zinc during pregnancy than in controls. The study suggested that this could be due to the disruption of skeletal muscle fibre development during foetal development due to insufficient resources. This mechanism could also apply to children who suffered SAM in the first few years of life. I will measure calf and hip circumferences to assess peripheral mass, and also skinfold thickness on biceps and triceps to assess peripheral, subcutaneous fat.

1.1.4.4 Long-term Effects of Nutrition on Physical Function Outcomes

When exploring the long-term effects of a nutritional insult, such as SAM, I felt it was important to not only consider anthropometric and clinical markers, but also functional ones. Anthropometric and clinical markers don't always translate into functional impact. Exploring functional outcomes allows us to assess long-term effects which have direct bearing on a person's everyday life. Functional outcomes which may have been affected by a nutritional insult include physical function and lung function. Lung function is discussed in the next section. Physical function is a broad term that can be used to refer to strength, fitness, mobility, balance, coordination and flexibility[137]. For the purpose of this study physical function refers only to muscle strength and physical fitness, as these are the areas most relevant to SAM-survivors, as well as having important implications for chronic diseases in adulthood and major implications for productivity and earning-potential[96, 138].

One of the most relevant and widely studied indicators of physical function is physical activity. Physical activity has been highlighted as an indicator of overall well-being in children and a

possible determinant, as well as output, of both their cognitive and motor development[139, 140]. It is also associated with better immunity and survival[96]. There have been studies which have found physical activity in SAM patients and SAM-recovering patients to be lower than other hospitalised children[141]. It is hypothesised that this is due to the need to balance energy requirements, either due to lack of calories, or spending more calories on weight-gain. There have also been a limited number of studies of physical activity in stunted children and teenagers, that have found mixed results [142, 143]. One study found that levels of physical activity of school aged children are unaffected by nutrition levels, whereas another found evidence that stunted pre-school children have lower physical activity than well-nourished ones [144, 145]. One hypothesis is that school-aged children are more subject to peer-pressure and therefore use their energy on physical activity, leaving less available for linear growth[144]. No studies to my knowledge have considered physical activity in SAM survivors several years post-discharge. There is mixed evidence regarding the long-term effects of prenatal malnutrition on physical activity, with both high birth weight and LBW being associated with low physical activity in later life[146, 147]. However, in these cases the inactivity is also associated with obesity, and whether obesity causes inactivity or inactivity causes obesity is not clear[148]. Based on evidence that LBW and some stunted children have reduced physical activity, SAM-survivors may also have this impairment. Hence, this study will assess physical activity in SAM survivors using accelerometers. A large variety of methods have been used in previous studies to assess physical activity, such as heart rate monitoring, questionnaires and observation of activity levels, however none of these are considered particularly accurate[149]. The doubly labelled water (DLW) technique is considered the gold standard for measuring energy expenditure under free-living conditions, however the Actigraph accelerometer has been proven to correlate well with DLW energy expenditure, hence for feasibility and logistical ease, this method was used in this study[149].

Physical capacity, also known as exercise capacity, is another important indicator of physical function. An individual's capacity for exercise is measured by their ability to endure exercise and/or the maximum workload achieved during the exercise period. Physical capacity provides information on the functioning of several body systems, including circulation, cardiovascular, pulmonary, muscle action and neural function; small deficits in one or some of these areas may only be observed during strenuous effort such as exercise[150]. The gold standard test of physical capacity is a Cardio-pulmonary Exercise Test (CPET) which measures electrocardiology (ECG), blood pressure, oxygen saturation and expired gases using a facemask during exercise on a cycle ergometer. However the availability of specialist laboratory facilities, equipment and trained staff to undertake CPET testing is not feasible in many places, hence many opt to use a variety of other "field-friendly" exercise tests[151]. Studies looking at physical activity often also consider

physical capacity; hence the evidence is similar to that mentioned above. There is evidence that underweight adults (low weight for age and/or weight for height) have reduced physical capacity, which is usually discussed in relation to capacity to work and productivity. Causality of this association has been implied by improvements seen in work capacity following weight-gain [152, 153]. Changes in body composition, such as increase in lean mass, are also associated with greater physical capacity and physical activity[154].

One study has also considered the long-term effects of chronic malnutrition on physical capacity by studying teenage boys in India (age 14-17, n=96) who had low HAZ at the age of 5 years. Physical capacity was measured as heart rate at a known level of physical exercise on a bike ergometer. They found a correlation between anthropometry at 5 years and that at 14-17 years. Physical capacity was correlated with current weight and height, as well as physical activity levels measured by observation over 2 days. Weight was particularly relevant for both physical capacity and physical activity, accounting for 64% ($p < 0.00\ 1$) of variance in physical capacity. Boys who were stunted at age 5 had lower physical activity and had a higher heart rate to achieve the same work capacity as other boys[155]. However, as malnourished children continued to be malnourished teenagers, this study could not conclude whether malnutrition in childhood affects physical function beside reduction in body size (i.e. if weight and height catch-up, is physical capacity still impaired?). Some studies proposed that the physical capacity of undernourished children, when expressed as a function of weight, or adjusted for height, approach or even surpass performances by European children, suggesting that deficiencies are directly as a result of weight and height limitations[156, 157]. Evidence from Chinese Famine survivors found those exposed to famine both prenatally and postnatally were significantly more stunted and had reduced work capacity resulting in lower economic outcomes. Work capacity was measured as total and home gardening hours worked; and agrarian, farm, and home gardening income. Effects were substantial; annual per capita agrarian income was reduced by 33% in famine survivors compared to controls[158].

The third and final measure of physical function to be considered is muscle strength. Muscle strength is clearly relevant to SAM as acute malnutrition results in loss of muscle (i.e. wasting). Whether muscle weakness persists several years after recovery from SAM has not yet been studied. The most common measure of muscle function is hand grip strength which is simple, non-invasive and a good marker of muscle strength of upper extremities. Hand grip strength is associated with both short and long-term morbidity and mortality, and is often used as an indicator of current nutritional status in hospitalised adults as it is easy to measure and muscle function reacts early to nutritional deprivation[159]. As well as nutritional status, muscle strength is likely to be related to lean mass quantity and quality, particular lean mass on the arms, quantity

of which can be measured by arm muscle area (AMA)[142]. Muscle strength is also likely to be linked to levels of physical activity. One study which looked at hand grip strength and AMA, as well as physical activity and physical capacity in relation to malnutrition, is by Benefice et al., (1992). 100 children aged 9-14 years were recruited in rural Senegal; those with WAZ <-1 were categorised as wasted. Wasted children were found to have lower AMA, ran slower, jumped less far and had weaker hand grip strength, however there was no difference in normal physical activity. HAZ also had a moderate influence on AMA and grip strength[142]. Although physical activity was unaffected by HAZ and WAZ in this study, it is important to note that it was measured by monitoring heart rate over 6 hours; heart rate can be influenced by infection, anaemia and emotions, therefore may not be an accurate reflection of physical activity.

The evidence described above tells us that both undernutrition and stunting can affect physical function; however we do not know whether an episode of SAM 7 years previous can have enduring effects in this area. Mechanisms which could link SAM with long-term deficits in physical function include lack of catch up growth since the episode, altered body composition and/or impaired muscle development. Weight, height and body composition have all been implicated as important for physical function; if SAM survivors are stunted and have a lower proportion of lean mass, as I have hypothesised, this could have subsequent implications for physical function. Equally, if the "thrifty phenotype" hypothesis has affected SAM survivors and resources have been prioritised to core body growth rather than limbs, this could result in less muscle mass on arms and legs, long after recovery. Lastly, Iannotti et al., hypothesised that prenatal nutrition could affect skeletal muscle fibre development which would have lasting effects on muscle mass[57]; this could also affect SAM survivors as muscle development occurs not only prenatally, but cell proliferation of the musculoskeletal system also undergoes rapid development between 2-4 years of age[109]. Disruption of muscle development could have long-term implications for physical function.

I hypothesise that compared to controls, SAM survivors have lower levels of physical activity, lower physical capacity during an exercise test and weaker hand grip strength. If one or all of these are true, this could have serious implications for NCDs in later life, as well as motor and cognitive development during younger years. Exploring the link between SAM and physical activity could contribute evidence towards a pathway linking changes in growth and body composition to an immediate functional output, and may be a possible link between early SAM and NCDs in later life. Although this study may not give us all these answers, establishing whether there is a link between SAM and later physical activity is a good starting place for future research.

1.1.4.5 Long-term Effects of Nutrition on Lung Function Outcomes

Lung function is another functional output, besides physical activity and muscle strength, which may have been affected by SAM in the first few years of life. It is not just cigarette smoking that causes adult lung disease, there is growing appreciation that the origins of some adult lung disease arise in early life [32, 160]. Not only could SAM affect lung function in childhood, but it could predispose individuals to developing chronic respiratory diseases in adulthood, as both asthma and COPD are pivotal elements to the DOHaD theory. Lung disease is a growing public health problem with COPD predicted to become the fourth largest cause of mortality by 2030 and the global burden of asthma predicted to rise as high as 300 million people worldwide[161, 162]. Evidence contributing towards the understanding of all the determinants of lung diseases in developing countries, both in childhood and adulthood, will aid in the design of holistic public health interventions.

Normal lung development begins in the 4th week of gestation and continues for the first few years of infancy[32]. From 16 to 27 weeks gestation the peripheral airways form and grow and the blood-gas barrier thins. Adults have between 300 and 600 million alveoli; alveologenesis begins at around 29 weeks gestation and is thought to continue until between 2 and 4 years of age, although there is no certainty about when alveoli cease to multiply [163, 164]. After birth, lung size increases with body size and there is a 30-fold increase in lung volume and a 20-fold increase in gas-exchanging surface area from birth to maturity[32]. An insult to growth and development during any of these time periods could feasibly damage lung function.

Many physiological and epidemiological studies, as well as animal studies, have shown that insults during intrauterine growth or early postnatal life are associated with decreased lung function and increased susceptibility to respiratory disease in childhood and later life [165-169]. There is already strong evidence that prenatal conditions, such as maternal smoking, prematurity, and LBW, are risk factors for later COPD [49, 74, 170]. Postnatal risk factors for which there is evidence of long term respiratory consequences include formula milk feeding, vitamin A, D or E deficiency, exposure to environmental pollutants and respiratory infections in childhood [32, 74]. One study concluded that the impact of some postnatal risk factors on adult lung health is as large as that of heavy smoking, and that an increased focus on these risk factors in early life could contribute to the prevention of COPD [74]. A recent literature review in The Lancet outlines pre- and postnatal early risk factors associated with adult COPD in more detail[160].

The specific aspects of lung function which can be affected by early risk factors have been described by several studies. A recent study found that school age children who were very LBW have lower expiratory volumes, and a greater portion of air remaining in the lungs after exhale (residual volume) than normal birth weight infants, which may be explained by collapse of the

small airways during exhalation due to a loss of lung elastic recoil and impaired alveolar function. Disruption of alveolar development due to adverse uterine conditions could feasibly result in a reduction in the number of alveolar attachments, and this is associated with a reduction in lung elastic recoil[171]. Balinotti et al. reported that very premature infants compared with controls born at term had significantly lower pulmonary diffusing capacity; again, it is suggested that a disruption to alveolar development could be the cause of this as it can result in a reduction in gas-exchange surface area[172]. Based on evidence such as these described as well as on the theory of lung development stages, I hypothesise that an episode of severe acute malnutrition in early childhood could result in children having reduced lung function. This could be due to fewer alveoli, thicker blood-air barrier, reduced lung weight, and/or impaired alveolar function although the mechanism will not be assessed in this study. I also hypothesise that those who suffered with SAM in early childhood (i.e. before 2 years) are likely to have more of an impairment than those who have SAM later as the majority of alveoli development happens before this time. However, it is possible for SAM sufferers of all ages to have long-term implications as it has recently been postulated that alveoli might continue to increase in size, number and complexity through to adolescence and even adulthood [173]. Although this postulation widens the potential period of vulnerability, it also offers exciting prospects in terms of recovery from early insults. If a deficit in lung function is seen in this cohort of SAM survivors, it could be possible, as with growth, that adolescence is a key time for intervention in order to reverse some of the long-term effects of SAM.

As with the other outcomes explored by this study, very few studies have explored the effects of post-natal undernutrition specifically on long-term lung function. One recent study considered women who were exposed to the 1944-45 Dutch famine between the ages of 0 and 21years and found that risk of hospitalization from obstructive airways disease, COPD and asthma in adult life was increased[174]. Risk of hospitalization from asthma was 112% higher among severely famine exposed women compared to unexposed; risk of hospitalization for obstructive airways disease was 57% higher. By separating analysis into ever-smokers and never-smokers, this study suggests that women exposed to famine in early life are more sensitive to the toxic effects of smoking. This could be an important consideration for developing countries who could be suffering a higher burden of chronic lung diseases due to the combination of smoking and undernutrition in childhood. Interestingly, the Dutch famine study found no difference in outcome between the different ages at which children were exposed to famine (0-21 years), despite the majority of lung development taking place in infancy and early childhood. This study does however have some methodological issues: recall bias, passive smoking was not controlled for, and there was a large loss-to-follow up. Another study, although using dated undernutrition and lung function parameters, found that wasted school children in India have worse expiratory flow rates than other

Indian children, but stunted children have largely unaffected flow rates[175]. Ong et al., found a similar correlation between wasting (low MUAC) and spirometry outcomes in another study of Indian children [176]. Finally, another study found observational evidence suggesting that children (5-18 years) of the Fulani tribe, who are more stunted and underweight than other Nigerian children, have significantly worse lung function outcomes than other Nigerian children. They suggest that the diminished lung function in the Fulani group could be attributed to respiratory muscle weakness or an overall deficit in energy, rather than disrupted alveolar development [177]. Neither the India studies nor the Nigeria study consider children who were malnourished in early childhood and their long term outcomes, they focus on children who are malnourished at school age and malnourished at the time of testing.

There is a need for more human evidence regarding the long term lung function outcomes of postnatal undernutrition. Particularly lacking is any evidence regarding the long-term effect of a SAM episode on lung function. This study aims to add to this currently limited evidence; I hypothesis that compared to controls, SAM survivors will have impaired lung function. I also hypothesise that younger SAM sufferers will having greater lung function impairment due to a greater disruption to lung development.

1.2 Hypotheses, Aims and Objectives

1.2.1 Aim and Objectives

The aim is to explore long-term (7 year) outcomes of severe acute malnutrition on growth, body composition, and functional outcomes in children.

Using a longitudinal cohort study design with sibling and community controls for comparison, the study objectives are:

1. To quantify the effects of an episode of SAM on growth and nutritional status 7 years post-discharge
2. To quantify the effect of an episode of SAM on body composition 7 years post-discharge
3. To quantify the effect of an episode of SAM on functional outcomes 7 years post-discharge, specifically:
    a) Physical activity
    b) Physical capacity during an exercise test
    c) Muscle strength
    d) Lung function
4. To quantify the effects of age at admission, severity of stunting and wasting at admission, time taken to recover, and presence of oedema at admission on each of the long-term outcomes.

1.2.2 Hypotheses

1. Compared to controls, SAM survivors have impaired linear growth (specifically (a) HAZ (b) shorter leg length) but (c) preserved head circumference
2. Compared to sibling controls, linear growth velocity is continuing at normal rates resulting in no catch-up growth since 1 year post-discharge.
3. Compared to controls, SAM survivors have an altered body composition, specifically (a) greater proportion of central fat to peripheral fat, (b) a lower magnitude of lean mass.
4. Compared to controls, SAM survivors have (a) lower levels of physical activity, (b) lower physical capacity during an exercise test and (c) weaker hand grip strength.
5. Compared to controls, SAM survivors have impaired lung function (namely reduced Forced Expiratory Volume in 1 second (FEV_1), Forced Vital Capacity (FVC) and/or FEV_1/FVC ratio z scores). Additionally, those who suffered SAM at a younger age (<2 years) have greater lung function impairments.

2 Chapter 2: Settings, Participants and General Methods

This chapter will summarise the setting, subjects, equipment and overarching methodologies for conducting the study. As this thesis is divided into 4 areas of exploration: growth, body composition, physical activity and lung function; methods specific to these areas will be presented in the relevant chapter.

2.1 Study Design

ChroSAM (Chronic Disease Outcomes following Severe Acute Malnutrition study) is a longitudinal cohort study with the late addition of sibling and community controls. The cohort originally included all patients admitted to "MOYO House" nutrition ward, Queen Elizabeth Central Hospital, Blantyre, Malawi, from 12th July 2006 to 9th March 2007. The original cohort was for the "PRONUT" study, a Randomised Control Trial (RCT) of pre/probiotics whose main finding was no effect on treatment outcomes[88]. This was followed by "FuSAM", an observational study at 1-year post-discharge[97]. This study (named "ChroSAM") builds on these previous studies, using already extensive baseline data for case children and the addition of sibling and community children as controls (2 controls per case child). Due to some debate regarding the most appropriate control group for nutrition follow-up studies[89, 178], this research will use both sibling and community controls. This should help distinguish between some of the community, household and genetic confounders. This is the biggest and most comprehensive nutrition cohort available in Malawi, and likely one of the biggest in the region.

2.2 Setting

The research described in this thesis is set in Blantyre District in Malawi. Malawi is a densely populated, landlocked country in southeast Africa. It has a national population of 16.8 million people, ranking it among one of the world's most densely populated countries. It has a GDP (nominal) per capita of USD$226, ranked the lowest in the world by most estimations[179]. Around 80% of the population live in rural areas and agriculture accounts of one third of GDP. The performance of the tobacco sector is key to short-term growth as tobacco accounts for more than half of exports. The economy depends on substantial inflows of economic assistance from the IMF, the World Bank, and individual donor nations, although much of this ceased in 2014 due to allegations of corruption. Population health, although improving, is still lagging behind much of the world with a national life expectance of 59.9 years and infant mortality of 48 per 1000 live births. 10.8% of adults have HIV, which is the 9th highest country prevalence in the world[180]. Additionally, 13.8% of children under 5 are underweight and 4% suffer from SAM[98]. The number of children affected by SAM regularly peaks during the rainy season (also called the 'hungry' season) when many families have to ration what little food is left over from last year's harvest, plus prevalence of malaria and diarrhoea is high, resulting in many children already suffering from chronic undernutrition, slipping into acute malnutrition.

Blantyre is Malawi's second largest city, with the population representing a 5.1% share of the national population[181]. The last official population data was collected in 2008 when the population of Blantyre stood at 661,256 people, with a growth rate of 2.8%. The city is estimated to have 1,068,681 inhabitants in 2015. There are also 338,047 people estimated in "rural Blantyre". Over 65% of the city's population lives in informal settlements which occupy about 23 percent of the land in Blantyre. The average population density in 2008 was 3,006 per square kilometre[181].

Blantyre has 18 government-run health centres and 1 government-run hospital: Queen Elizabeth Central Hospital (QECH), which is the largest referral hospital in the country and also the teaching hospital for the national College of Medicine. Besides these, the health system also has 3 private hospitals, many private clinics, clinics run by NGOs and religious organisations, and numerous traditional healers. "Moyo" nutrition ward (here after referred to as Moyo ward) is located in the paediatric department at QECH and is one of 3 nutritional rehabilitation units (NRU) in Blantyre District. Moyo means "life" in Chichewa, Malawi's national language. Since the full roll-out of Community Management of Acute Malnutrition (CMAM) in Blantyre in 2008, case numbers have dropped considerable at Moyo ward and the characteristics of cases has also changed. Presently, the majority of cases are referred from health centres or other wards, and almost all cases have complications in addition to SAM. Cases of SAM without complications are

treated by CMAM programmes at health centre level. QECH and Moyo ward are part of the government health system and therefore all treatment is provided free of charge.

Between 2006 when the baseline data for this cohort was collected and this current follow-up in 2013-14, both the national and international guidelines for treatment and management of SAM have changed considerably. In 2003, Malawi was pioneering the development of CMAM and Moyo ward in particular was key in developing and validating a new, locally-manufactured Ready-to-use Therapeutic Food (RUTF)[182, 183]. In 2006, cases were being treated on the ward with RUTF (which replaced F100 milk in transition phase in 2005) and rehabilitation was conducted in an outpatient manner, with children returning to Moyo ward for review and RUTF collection. As of 2008, a full CMAM programme began, which meant many of the cases previously being treated at Moyo ward began being treated by health centres using an outpatient model, and those children who were treated on Moyo ward continue their rehabilitation in a CMAM programme post-discharge, without returning to the ward for review. Considering these changes have taken place over the course of the cohort, it is important to outline which admission criteria and treatment strategy was applied to this cohort in the following section.

Image 1: Map of Africa with Malawi highlighted, and image of mothers sitting outside "Moyo ward" nutrition rehabilitation centre at Queen Elizabeth Central Hospital in Blantyre, Malawi.

2.3 Study Participants

2.3.1 Eligibility Criteria:

- All children admitted to 'Moyo' ward from 12th July 2006 to 9th March 2007 ("case") and
- A sibling closest in age to a "case" child and
- A child living in the same community as a "case" child, who is also the same age (within 12 months) and gender as a "case" child
- All control children must be older than 4 years of age and younger than 15.9 years of age, for better comparison between the groups
- Children must have fully informed consent from a parent or guardian, and assent if they are older than 13.
- Children should be living in locations that can be visited from Blantyre in a day-trip (this therefore excludes those who have moved to Lilongwe, Mzuzu and areas beyond Mangochi).
- Children should be located within a maximum of two attempts of searching for them

2.3.2 SAM Admission Criteria and Treatment

All "case" children in this study were admitted to Moyo ward with SAM. The Moyo ward admission criteria, following Malawi National Guidelines[184] and consistent with international guidelines[185] at that time, for the case definition of SAM were:

• Weight-for-height <70% of median (NCHS reference)

or

• Mid-Upper Arm Circumference (MUAC) <11cm

or

• Kwashiorkor (oedematous malnutrition)

CMAM was started in Blantyre district in 2008, so the distinction into complicated or uncomplicated SAM was not applicable. All patients identified as having SAM were admitted for initial inpatient treatment. Children presenting at QECH were first assessed for weight and clinical issues at A&E, and then referred to Moyo ward if suspected of SAM. Children were sometimes also referred from other wards following stabilisation of other clinical issues. Once at Moyo ward, children underwent more thorough anthropometric (weight, length and MUAC) and clinical assessment, and nutritional and clinical treatments began.

Nutritional treatment followed the standard 3-stage approach:

I. *Stabilisation Phase*: Children were fed F75 therapeutic milk every 3 hours, including throughout the night. The quantity of milk was determined based on the child's weight using standard charts from the Malawi National Guidelines[184].

II. *Transition Phase*: to progress to this phase, children should show clinical improvements and return of appetite, as indicated by easily finishing all F75 milk prescribed in Stabilisation phase. At this phase, RUTF was introduced to the diet, consistent with the then-new CMAM guidelines[186]. 200 kcal energy, per kg body weight, per day was prescribed in the form of RUTF, in addition to the F75 milk ration. This ensured that children who were not ready or not able to finish the ration of RUTF would still receive enough calories as F75 was still provided.

III. *Rehabilitation Phase*: When easily eating at least ¾ of the RUTF ration, the child was deemed to have an active appetite and could therefore move into Rehabilitation Phase, provided they were also clinically well. This meant that they could be discharged for treatment at home at the next opportunity (discharges from the ward took place twice per week). They were provided with a 2-week ration of RUTF, F75 was stopped, and other foods and fluids were allowed. Children returned to Moyo ward after 2 weeks to pick up further rations and receive a clinical assessment. For children in this cohort, transport money was reimbursed in order to minimise defaulting.

Children were discharged from Moyo "OTP" (outpatient treatment programme) following 2 consecutive visits at or above target weight of 80% median weight-for-height. Minimum time on the Moyo OTP was therefore 4 weeks. Maximum time was 10 weeks; if the child had not reached and maintained their target weight after this time, they were deemed a "nutritional failure" and referred elsewhere. Common referrals from Moyo OTP were to HIV and disability services. All patients discharged from Moyo OTP were referred to their nearest supplementary feeding centre for a further 4 months supplementary feeds (using corn-soy blend) in order to minimise the chances of relapse. These were located at health centres distributed throughout the district.

HIV testing and treatment was also an important part of Moyo ward treatment procedure. HIV prevalence on Moyo ward at the time of recruitment (2006) was over 40% (ref FUSAM or Markos PhD). An opt-out policy of HIV testing and counselling was offered to all patients in order to facilitate timely referral to HIV treatment services. HIV seropositive patients were treated according to national protocols; referral for antiretroviral (ARV) medication was based on clinical staging criteria and CD4 count if available. An ARV waiting list in 2006 meant that additional ARV therapy rarely started during inpatient phase of nutrition treatment. All HIV positive patients were started on long term co-trimoxazole prophylaxis as soon as diagnosed.

2.3.3 PRONUT and FuSAM Studies

The cohort which forms the "case" children for this study was initially created for recruitment of participants into two previous studies: PRONUT study and FUSAM study[88, 97]. See **Figure 3** for summary flow-chart of previous studies. PRONUT was a randomised, double-blinded, placebo-controlled trial exploring the effects of a pre/probiotic (Synbiotic2000 Forte™) on treatment outcomes in children affected by SAM, which took place in 2006 and 2007. The study found no difference in nutritional or clinical outcomes between the intervention and control groups. This meant that the control and intervention groups could be merged into one cohort, and the group was then studied as a whole during the FuSAM study.

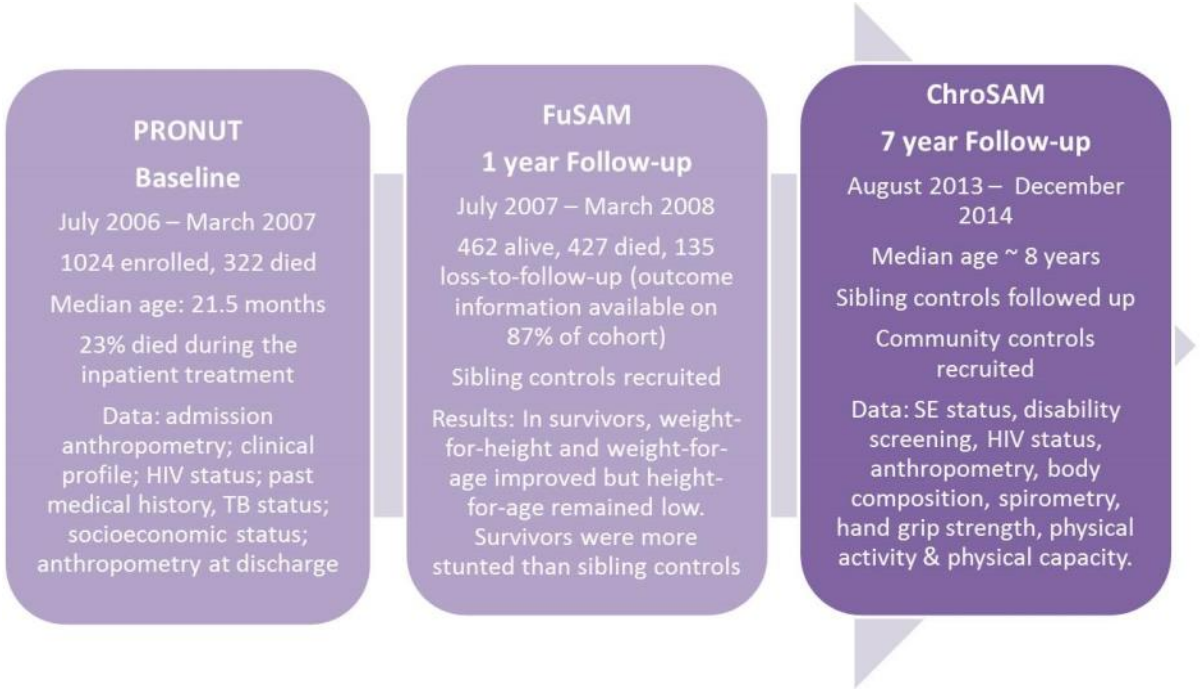


Figure 3: Flow diagram outlining the 3 studies using the Malawi SAM cohort

FuSAM aimed to describe long-term outcomes following SAM and identify key mortality risks factors. The study followed-up all children thought to be alive at 1 year post-discharge; it found that 10% of the cohort died between discharge and 1 year post-discharge, and Cox regression identified HIV, low weight-for-height, low MUAC and low weight-for-age as major risk factors for death ($p<0.001$)

The majority of "case" children in this study have previously taken part in both PRONUT and FUSAM studies, although not all. Those families that declined to take part in the PRONUT and/or FUSAM were still approached for this research. Additionally, those children who could not be located during FUSAM study where still searched for during this research, with some success.

Although PRONUT and FuSAM concluded that there was no significant effect of synbiotics on nutritional or clinical outcomes, PRONUT study post-hoc analysis did observe a reduction in outpatient mortality amongst the synbiotic intervention group. For this reason, this study (ChroSAM) will assess for any long-term differences between intervention and control groups among the case children before any further analysis.

2.3.4 Locating Participants

Each case child (i.e. all ward admissions from 12th July 2006 to 9th March 2007) has a paper file which is stored in chronological order by date of admission (**Image 2**). If the child died on the ward or was found to have died at the one-year follow-up (FUSAM), these folders are red and orange respectively, and were not included in this study. In order to locate study participants, blocks of 50 files were sorted into geographical regions using the verbal map in the file, starting from the first admissions in July 2006. The "verbal maps" in the files where first collected during ward admission, for PRONUT study. The maps describe how to find the child's home, as provided by the carer of the child, and usually refer to prominent landmarks such as towns, schools and churches, and usually include prominent people in the community who can direct the study team. Here is an example of a typical, fictional "verbal map":

> *"Soon after Phalula town centre on the main road to Lilongwe, there is a right turn where they sell Makala (charcoal). Take this road, cross the bridge and look for Chigdi primary school on your left. Take the road behind the school and continue until you reach Kasamba maize mill. There ask for Chapola plot. This family also go by the name Tiyesembo."*

Taking of effective maps was aided by a large scale, physical map held on the ward which enabled staff and carers to identify local landmarks on route to the patient's home. Some maps were updated during the FUSAM 1-year follow-up with additional details. A few files do not have a verbal map, if the family defaulted from the ward before it was collected, or if the family declined to provide it. Some files have GPS coordinates so the location can be looked up using Google Earth. Each geographical area was then visited with the aim of locating 2 – 3 cohort children per day. When these 50 files have been visited, the next 50 are selected and sorted. Ideally, the children would be visited in exact order of admission, however the need to group the children by geographical area in blocks of 50 files is necessary in order to minimise travel time and maximise resources. Two of the study nurses were part of the team who worked on PRONUT and FUSAM, so they were familiar with some of the locations and communities, despite there being a 6-year gap since the previous follow-up.

Image 2: case children's files stored in chronological order by admission date; blue files were last known to be alive, red died during admission, orange died post-discharge and yellow declined to take part in "PRONUT" study. The image on the right shows that each file has a label with the patient name and study ID number

Once the case child was found, the sibling control could also be identified and located. In order to locate the community control child using random selection, a bottle was spun at the house of the case child and the study team walk in that direction. They enquire at all houses in that direction until a child of same age and gender as the case child, who's family are willing to consent, is found. If the area was particularly rural, it was necessary to ask the case family if they know of a suitable community control child, and in these cases a note was made on the file that this was 'convenience selection'. If a household member was found but the child was not at home, this child can still be recruited and invited to a hospital appointment.

2.3.5 Informed Consent Process

Once the family of a case child has been located, the main carer was identified and the study explained to him or her using the standardized information sheet. In most cases, the main carer was female, and informed consent sought from her in order to enrol her child (see English copies of the information sheet and consent form in **Appendix 1**). Additional consent from the male head of the household and the village chief was sought if possible, as this allowed the female carer and child to take part in the study in the confidence that she is supported by her family and community leaders. If all parties understood the information sheet and were happy to participate, the carer signed the consent form. If the child was 13 years or older, assent from the child was additionally sought before proceeding. If the carer was illiterate, there was an impartial witness to help the carer in the consent process, ensuring that the consent form had been correctly read to and understood by the consent-giver. Once consent was given, the data collection began with questionnaires and anthropometry at the child's home. A hospital appointment date was then

given to the family for data collection to continue at the hospital. If the family and/or child did not want to consent after the study had been fully explained, then a note was made on the child's file and they were not approached again.

For sibling controls, if the child was 13 years or older, they had to assent to take part. If the subject was 18 years or older, they were able to fully consent or decline for themselves without the input from their guardian. If the child was older than 13 and did not assent, then I asked the sibling next closest in age to take part. If the case child had no living siblings, or none willing to consent, then no sibling control was enrolled.

For community controls the same information and consent process took place as with the case family. If the family and/or child did not want to consent after the study had been explained to them, another appropriate household was sought. On a few occasions, in small villages, no appropriate child that was willing to consent could be found.

On most occasions, at the request of the carer, the study team made a visit to the child's school in order to seek permission from the head teacher for the child to miss a day of school in order to participate in the research. In some cases, if the school was far away or if it was not open that day, a letter for the head teacher was given to the carer to provide to the school as an explanation for the child's absence (see letter in **Appendix 2**).

2.4 Ethical Approval:

Conditional ethical approval was granted for the research project as a whole by Malawi College of Medicine Research and Ethics Committee (COMREC) in 2013, and fully granted in 2014 (see **Appendix 3** for letters from COMREC and the approval certificate). Additionally, Ethical approval was granted by University College London (UCL) Research Ethics Committee in 2013 (see **Appendix 4** for letters of approval from UCL).

2.5 Sample Size

The sample size was determined by the number of survivors from the original cohort and the number which we are able to find and recruit. Prior to data collection I estimated our sample size to be around 960 children (400 cases, 320 sibling controls, 240 community controls) based on success rate of the 1-year follow up[97]. For each of the main variables I conducted sample size calculations to assess whether these numbers were adequate for detecting a significant difference, powered at 90%; these calculations are presented in relevant method sections in following chapters. Statistical power is the likelihood of detecting an effect if one does exist. Greater power reduces the chance of a type II error. As HIV is a key confounder across all outcomes, it was desirable for the target sample size to be greater than that required for simple analysis in order to allow for analysis of subgroups. In reality, I recruited 320 cases, 217 siblings and 184 community

controls; slightly below expected but still adequate for majority of analyses. It is also important to note that one major goal of this project was to inform sample size calculations of *future* intervention studies; hence the inflexibility of our sample size for this study was not a major concern.

2.6 Data Collection Overview

The families and children in this study participated in a wide variety of tests and measurements. Not all of these variables are included in this PhD but they are relevant to other sub-studies, and all relate to the long-term effects of acute malnutrition. Those questionnaires and measurements which are not included in this PhD are highlighted in grey.

Field Measurements (at the child's home):

1. Questionnaires
    a. Basic information and general health
    b. Socioeconomic status (SES)
    c. Domestic violence
    d. Maternal mental health
    e. Family health
    f. UNICEF Disability screening
    g. DUREL Faith views
2. Basic anthropometry
3. Family anthropometry (all siblings and both parents)
4. Accelerometers fitted

Hospital Measurements:

The following measurements took place if/when the children attended their hospital appointment, previously arranged at their home. The whole battery of assessments took around 5 hours, and generally took place in the exact order listed:

1. Accelerometers collected
2. Blood drawn (full blood count, lipid profile, HbA1c, metabolomics, and fasting glucose)
3. Oral glucose tolerance test – subset of 100 children
4. Saliva collection for salivary cortisol
5. Remaining anthropometry
6. Skinfold thicknesses
7. Bioelectrical impedance analysis (BIA)
8. Blood pressure and oxygen saturation

9. Maternal blood pressure
10. Handgrip strength
11. Spirometry
12. MRI scan – subset of 50 case children
13. CANTAB cognitive test
14. iStep exercise test
15. Retina photos

2.6.1 Staff Training

The research team consisted of 2 full-time nurses and 4 full-time field workers, plus some assistance from the 5 nurses who work on the malnutrition ward. All full-time staff members were trained on all aspects of data collection, however, some team members became specialised in certain tests in order to aid with consistency of testing. For example all members of the team were trained in conducting spirometry yet the majority of tests were conducted by me or 2 of the field workers.

Intensive training of study staff, including myself, was required for each of the measurements. For spirometry, I received 2 weeks intensive training at UCL PORTEX Unit, Great Ormond Street Children's Hospital. I and the Chichewa-speaking team also went out on a field trip with the Blantyre Health Study (BHS), who were conducting spirometry in adults in Malawi, in order to get some tips on how best to explain the process in Chichewa.

All team members, including myself, were required to attend and pass a 'Good Clinical Practice' (GCP) course, which covered topics such as ethical research procedures and the informed consent process. During training on specific questionnaires, the whole team explored the pilot data collection forms and questionnaires and had the opportunity to feedback suggestions for amendments. Study staff training was conducted from March - July 2013. Refresher training then took place in January 2014, and further refresher training took place in April 2014, as a new team member joined the group.

For anthropometry, following training by the Nutrition research department at Institute for Child Health, a "Standardization session" took place whereby each team member had to measure 10 pilot children twice. This allows for comparison of intra-operator and inter-operator differences, and highlighted which measurements needed improvement or more training. The greatest intra and inter –operator differences occurred on hip circumference and waist circumference measures.

2.6.1.1 Anthropometry Standardisation Session Results

Following training, I conducted an anthropometry "standardisation session" with the aim of extensively practicing the measurements as well as identifying inaccuracies with specific measurements or individual team members. This process involved 8 children whose anthropometry was measured twice by each trained data collector. The session took place at our data collection room in the hospital; children between the ages of 6 and 11 were recruited from siblings of hospital admissions or children of nursing staff, they were not part of the ChroSAM study. The children were stationed around the room and the data collectors were split into pairs to move systematically from child to child conducting their measurements. This circuit was repeated after lunch so that each data collector measured each child twice. Anthropometric measures conducted were height, MUAC, bicep and waist skinfold thickness, head and waist circumference and chest width. These measures were selected to ensure practice with the whole range of equipment. Weight was not included as digital scales have negligible intra- and inter-operator error.

The intra-observer technical error of measurements (TEM) were calculated and compared (**Table 2**)[187]. TEM is calculated by finding the deviation between an operator's individual measures, squaring it, summing this for all the children measured, dividing this by number of children x 2, and then finding the square root, as shown in this equation:

$$\text{TEM}=\sqrt{\frac{\sum {d_i}^2}{2n}}$$

Where $\sum d^2$ = summation of deviations raised to the second power, n = number of children measured, i=the number of deviations

The accuracy for each measurement (rather than each individual operator) was considered using the mean standard deviation (SD) between all operators across each child (also presented in **Table 2**). The "gold standard" intra-observer TEM is also presented in Table 2; this is the TEM of the expert anthropologists conducting the training for WHO Multicentre Growth Reference study[187]. Consulting the results of this exercise and discussing them with the team allowed for identification of those needing more training on specific measurements, for example Operator 2 and MUAC. I also identified waist skinfold thickness and waist circumference as measurements with high SD where extra care is required when taking measurements.

	n (children)	Height	MUAC	Bicep skinfold	Waist skinfold	Head circ	Waist circ	Chest width
Operator 1 TEM	8	0.47cm	2.06mm	0.23mm	1.56mm	0.28cm	1.71cm	0.59cm
Operator 2 TEM	8	0.38cm	6.95mm	1.19mm	2.12mm	0.38cm	0.93cm	1.08cm
Operator 3 TEM	8	0.39cm	1.22mm	1.16mm	1.38mm	0.19cm	1.55cm	0.38cm
Operator 4 TEM	9	0.38cm	1.66mm	0.23mm	1.68mm	0.31cm	1.53cm	0.66cm
Operator 5 TEM	9	0.60cm	2.35mm	0.83mm	0.79mm	0.34cm	1.02cm	0.30cm
Gold standard TEM[187]	10	0.23cm	1.70mm	0.40mm (tricep)	\	0.12cm	\	\
Mean SD across children	9	0.57cm	1.70mm	0.84mm	1.90mm	0.69cm	1.21cm	0.62cm

Table 2: Intra-observer technical error of measurement (TEM) for anthropometry standardisation session conducted during ChroSAM team training, as well as "Gold Standard" TEM from WHO Multicentre Reference Study and mean standard deviation (SD) between operators across each child.

2.6.2 Pilot Phase

The pilot phase of this study took place in July 2013 with 10 case children from the actual cohort plus controls (timing of all study phases can be seen in Gantt chart in **Appendix 5**). This process informed many details of the study logistics, such as the need to approach fathers and chiefs for consent in addition to female carers. This phase also informed the average cost of transport required by study families and the need to carry petty cash on field visits as not being able to access transport money prior to travel was identified as a barrier to attending study appointments. I also learnt how long the hospital appointment takes, and saw the need to provide both a snack and lunch to the participants. The 'incentive package' was also discussed with the pilot participants and was found to be appropriate and acceptable.

During the pilot phase I also learnt that some of the participants did not like the accelerometers being red in colour, and I further learnt that spray painting them yellow was less convincing than a full explanation of their function! All other tests were deemed 'acceptable' to participants and their families, except the blood test which sometimes prevented people attending their appointments. The need to stress that all aspects of the research are optional, especially the blood test, was noted by the study team. The pilot phase also refined some of the Chichewa translation of the questionnaires and amended some of the skip-logic.

2.6.3 Data Recording and Management

The majority of data recording took place on Samsung Galaxy mobile phone devices using open-access ODK-based open-platform software called "CommCare ODK" (www.commcarhq.org). During the pilot phase and very beginning of the study, paper forms were used and these were

entered into the CommCare database using the mobile phones retrospectively (paper versions of forms can be seen in **Appendix 6**). Once I had correctly programmed the software, we used mobile phones to enter data at the point of collection, without the need for paper. I visited a research project run by Institute of Global Health (UCL) in Machinji, Malawi that was using CommCare software in order to learn about its application in the field. This electronic data collection method misses out the step of data entry at a later date which saves time and reduces the probability of data entry errors. The phones also have the benefit of on-the-spot calculations for anthropometry, particularly quality control of anthropometry measures, and ensure that the correct skip-logic is applied, with no relevant questions left blank. In order to keep track of which forms were completed in the field and which needed to be done at the hospital appointment, we use one paper form known as the 'Master Checklist' which required the team member to tick which forms they have completed. This paper form also had space for free-text notes, which are not as easy to enter using the mobile phones. Lastly the paper form had space for adding further details to the child's address or adding a new address so that the next follow-up which might take place is not reliant on access to the CommCare database for this information. Some data, such as blood test results, spirometry traces, CANTAB cognitive test scores and retina photos were stored on specially designed databases, rather than on CommCare.

Image 3: Photo of mobiles phones used for data collection and screen showing CommCare software highlighting an error during data entry

All data for each child was checked by me at the end of each week, before allowing the paper file to be returned to the filing cupboards. On occasion, children would be revisited at their home to gather missing data or clarify anomalous data. Any amendments needed to the data where

recorded as an instruction in a STATA do. file. Any clinical issues which arose during data collection were highlighted at the checking stage and the child invited to see the relevant clinician. The spirometry database was downloaded once per month, I conducted initial quality control conducted and then sent the data to the lung function department at Great Ormond Street Hospital (GOSH) for checking of queries and random checking of a further 40% of the spirograms. Following this, each spirometry database was exported into Microsoft Excel and compiled into one master spirometry database. The GOSH expert was blinded to the case/control status of the results. In general, the data collectors were not blinded to study group status due to lack of an adequate system which would allow for blinding but not allow potential errors in data recording; due to human resource limitations, all data collectors worked on all aspects of recruitment and data entry therefore could not be blinded.

At the end data collection, the majority of the data, which was on the CommCare database, was imported into STATA12 and the correction do. file run. After checking all the ID numbers for errors, the spirometry database was also imported into STATA12 and merged based on the ID number. Selected relevant data from the PRONUT and FUSAM database was also merged based on ID numbers to create one long database, where each child has their own row of data.

2.6.4 Demographic Questionnaires

The questionnaires were usually administered at the children's homes; in exceptions they took place at the hospital appointment. The questionnaires, all administered in Chichewa language by the trained field team, can be seen in **Appendix 7** (English and Chichewa versions). These questionnaires were developed based on those used in the "FuSAM" 1-year follow up study and adapted following consultation with the UCL research team, other researchers at Malawi-Liverpool Wellcome Trust in Blantyre, some members of the field team and relevant experts in specific areas. The following four demographic questionnaires where asked:

- **Questionnaire A: Basic information**
  Includes age, sex, alive/dead/sick, birth order, school achievement, and questions about hospital & outpatient visits, SAM admissions, history of TB, asthma, pneumonia, and HIV status. Questions are based on similar questionnaire for FuSAM study for continuity
- **Questionnaire D: Family Characteristics**
  Includes parent's marital status, occupation, literacy and education based on FuSAM questionnaire, as well as questions about domestic violence and social welfare based on Malawi DHS and select questions from Multidimensional Scale of Perceived Social Support (MSPSS) previously translated and validated in Malawi[188, 189]
- **Questionnaire E: Household and SES**
  Includes questions about household assets and water and sanitation facilities, based on Malawi DHS, as well as questions about tobacco and cooking smoke in the home adapted from the American Thoracic Society's ATS-DLD-78C respiratory questionnaire
- **Questionnaire K: Family health**
  Includes maternal and paternal HIV status and general health, and history of SAM in all of the non-study siblings, based on a similar questionnaire from FuSAM study for continuity

2.6.5 Maternal Mental Health SRQ

This questionnaire (**Appendix 8**) was asked to the main female carer of the study child, most commonly the mother. The 20-item yes/no Self-Reporting Questionnaire (SRQ) was designed by WHO to identify common mental disorders such as depression, anxiety, and somatic manifestations of distress in the community[190]. It has been translated and validated for use in many developing countries including Malawi, where a cut-off score of 7 or higher is recommended for identifying mental distress. For our study, this questionnaire was administered by a senior nurse who had previous experience using it; carers scoring more

than 12 or showing signs of suicide intent were referred to routine psychiatry services at QECH.

2.6.6 Disability Screening Questionnaire

Developed by the Washington Group on Disability Statistics in collaboration with UNICEF, this questionnaire was designed to be efficiently nested into larger surveys in order to collect broad information of disabilities in the community, as well as being a standardise tool for disability screening across all countries[191]. It includes questions on six core functional domains: seeing, hearing, walking, cognition, self-care and communication (see **Appendix 9**). I used this questionnaire to attain information on potential confounders as well as assess differences in disabilities across cases and control groups.

2.6.7 Assessing HIV Status

HIV status is an important variable as it is potentially a major confounder for many of the main study outcomes. HIV status was assessed firstly by ascertaining whether the participant had ever had a HIV test, followed by proof of the results of that test, usually in the form of a stamp in the individual's "Health Passport" (Data Collection Form A). If no test had been taken or no proofs of results were available, the participant was offered a HIV test during the hospital appointment. Many case children had proof of their status in their health passports as this is a routine test upon hospital admission; however a large proportion of community controls, sibling controls and parents had never been tested. If consent was given, the HIV test was administered by a field worker or nurse with a valid Ministry of Health HIV testing and counselling certificate, following National protocol. This involves two rapid tests, Detemine™ as a screening test and Uni-Gold™ as a confirmatory test; all results were delivered in a private place with appropriate counselling. For those who did not know their status and did not consent to a HIV test, HIV status was recorded as "unknown".

2.6.8 Assessing Puberty

As the children were already undergoing a long day of obtrusive tests and time was short, I decided not to rank puberty by Tanner stage or other physical assessment[192]. Puberty was simply recorded as yes/no/don't know by asking the guardian or individual if the child has started puberty yet. The cultural understanding of onset of puberty for girls is start of menarche; for boys voice breaking was suggested to carers as a sign of the onset of puberty. This was a rough guide in order to avoid invasive examination (Data Collection Form A).

2.6.9 Gestures of Thanks/Incentives

All families who attended the hospital appointment were given refreshments, a mid-morning snack, a hot lunch, reimbursement of the transport costs and a small incentive package worth approximately USD$2.50. This included soap, sugar, salt, matches, pencils, pencil sharpener and school exercise books. The children were also provided with stickers, lollipops and entertainment after various assessments; we tried to make the process as fun as possible for the children involved and their accompanying siblings.

2.6.10 Quality Control Systems:

Repeat Measures:

Anthropometry is notoriously difficult to measure accurately if repeat measures are not taken to ensure quality. Therefore, for all the anthropometric measures in this study (except weight), a quality control system which involves two members of the trained study team taking independent readings, based on that used in the WHO Multicentre Growth Reference Study, was used[193]. The mobile phone data collection devices were designed so that each of the two staff members entered their anthropometric measure, without knowing what the other had entered. The device then informed the operators whether or not the two readings were within the acceptable range. If the two measurements were similar enough, then the team moved on to the next measurement; if the readings were outside of the maximum allowed difference then both operators had to repeat the measures. If the team repeated the measurements 3 times and were still not within the allowed difference, then a third team member was asked to take a measurement. The team could not proceed to the next question until they had two readings within the acceptable range. The final value used in the results was the average of the final pair of readings. The WHO Multicentre Growth Reference Study found that large differences owing to reading or recording errors were resolved by using this method[193]. The maximum allowable difference for acceptable precision was developed by the WHO Multicentre Growth Reference Study based on the TEM obtained in their initial standardization session conducted in Brazil, which predicts a range that will only require re-measurement 5% of the time. The WHO study decided to widen the allowable range for skinfold thickness to 2mm because this was the measure which the participants found most uncomfortable[193]. Following my own standardization session I decided that we would decrease this maximum allowable difference for skinfold thickness readings to 0.5mm as this would still allow for a low percentage of repeat readings. Weight was not re-measured as digital scales have negligible intra- and inter-operator error when measuring older children. See **Table 3** for maximum acceptable differences for each reading.

Measurement	Maximum allowable difference
Standing Height	0.7 cm
Sitting Height	0.5 cm
Head Circumference	0.5 cm
Mid-upper arm circumference (MUAC)	0.5 cm
Waist circumference	0.5 cm
Hip circumference	0.5 cm
Calf circumference	0.5 cm
Chest circumference	0.5 cm
Chest width	0.5 cm
Chest depth	0.5 cm
Knee Height	0.5 cm
Skinfold thickness –left bicep	0.5 mm
Skinfold thickness –left tricep	0.5 mm
Skinfold thickness –left subscapular	0.5 mm
Skinfold thickness –left suprailiac	0.5 mm

Table 3: Maximum allowable differences between measurements conducted by independent data collectors; a quality control system developed by WHO Multicentre Reference Study.

Questionnaires were also repeated for 10% of participants as a quality control check. 10% of carers who had answered the study questionnaires during the field visit were asked the questionnaires a second time during the hospital visit, in order to assess intra-participant validity, and inter-operator validity (see results of questionnaire data quality in **Chapter 3**). Similarly, BIA readings were taken twice, one immediately after the other, in order to pick up any random errors on the part of the device, and additionally, a 10% of participants had their BIA reading taken again at least 2 hours after the first pair of readings, in order to validate quality of the readings, particular at different points on the same day (see results of BIA data quality in **Chapter 5**).

Validity question: During the pilot, field staff were concerned that some mothers did not fully understand all the questions in the questionnaires, particularly the disability screen questionnaire, or they didn't respond truthfully due to lack of interest. I therefore added a validity question at the end of some questionnaires, for the interviewer only, asking their opinion on the validity of the responses:

"In your opinion, are the responses to this questionnaire valid?" YES/NO

"If NO, why not?

1. Respondent didn't understand the questions
2. Didn't pay attention
3. Lack of motivation
4. Impatient and restless
5. Negative attitude toward the interviewer
6. Interview interrupted"

Age: Age is a key variable, particularly for calculating anthropometric and spirometric z scores. It is well known that age and date of birth can be poorly recorded, or poorly remembered by carers in many countries. Therefore, in order to minimise error, the carer was asked the child's date of birth, and the mobile phone data collection device calculated the child's age in years. The carer was then asked to confirm if this age was correct; inconsistencies or errors could therefore be immediately identified and further details could be asked to determine the child's true date of birth.

Calibration: Each instrument was calibrated according to the manufacturer's instructions. The following equipment was calibrated daily: Weighing scales using 5kg reference weight, Stadiometer and Body Callipers using 1m wooden stick, Skinfold Callipers using 4mm wooden cube, Spirometer by blocking airflow and therefore registering zero flow, and lastly BIA Quadscan machine using a metal calibrator provided by the manufacturer.

2.7 Data Analysis

2.7.1 Potential Confounders

Much controversy exists regarding proper methods for the selection of variables in confounder control. Many authors condemn any use of significance testing, some encourage such testing, and others propose a mixed approach[194]. This study used a mixed method. I deduced potential confounders from reading related literature and then creating conceptual frameworks/causal pathways for each of the main outcomes being assessed. A confounder is a third variable which is associated with both the outcome and the exposure and can distort the magnitude of the relationship between the two factors of interest if it is not properly accounted for. Whether this "third variable" has an association with the outcome of interest can be assessed with regression analysis; however statisticians advise that I cannot rely on statistical significance alone[195]. Choosing which confounders need adjusting for should be based on biological plausibility initially, followed by statistical significance testing. Selected potential confounders were included in the regression model, where available. See **Figure 4** Error! Reference source not found.for conceptual frameworks identifying potential confounders for each major outcome.

One potential confounder (or effect-modifier) which could affect all of the outcomes of interest in this study is HIV status. HIV is associated with the exposure (SAM): infections associated with HIV result in higher calorific demand resulting in a greater likelihood of all types of undernutrition; and HIV and resultant infections are associated with the outcomes: poor growth, dyslipidaemia, poor lung function, weaker muscle strength and lower physical activity levels[196-199]. As HIV is so closely linked to both SAM and some of the outcomes studied, not only has it been included in the regression models, but I have also conducted stratified analysis in order to observe the effects of SAM without the influence of HIV. Ideally, I would have liked to assess the effects of SAM with HIV as well by comparing HIV positive cases to HIV positive controls; however the sample size for HIV positive controls was very small.

Birth weight is also a potentially important confounder or effect-modifier. As discussed in the **Chapter 1**, many studies have found LBW to be associated with stunting, adverse body composition, poor lung function, and other risk signs of chronic diseases[32, 67]. LBW infants are also more likely to suffer from malnutrition[200]. Unfortunately, this is one of the major potential confounders which cannot be adjusted for in this study as accurate birth weights for participants are not known. Although at present most infants in Malawi are born in a health facility and their birth weight recorded, this was not the case when our study participants were born, and in instances where it was recorded, these documents have since been lost. A rough idea of birth weight was collected for case children at admission by asking mothers in the child was born normal weight, small or very small; this can be assessed as a predictor of outcomes in case

children and will therefore give some idea of the importance of birth weight on long-term outcomes, but this variable is not available for control children hence we cannot know if LBW is more prevalent among cases than controls.

HIV status, age, sex, and socio-economic status (SES) are included as potential confounders in all regression analyses in this study, regardless of statistical significance, as it is highly plausible that these variables are associated with all outcomes, independent of the exposure (SAM). Although controls were "matched" to cases by some variables (i.e. community controls were matched on age and sex; sibling controls were matched on "household environment", SES and genetic potential) these factors still require adjusting for during analysis as the matching process can create an association between the outcome (i.e. SAM) and a matched criteria (e.g. age) when there was no association before the matching was conducted[201]. Additionally, as analysis is not "matched" the differences in mean age, sex and SES between the groups will need adjusting for.

Stunting, particularly limbs

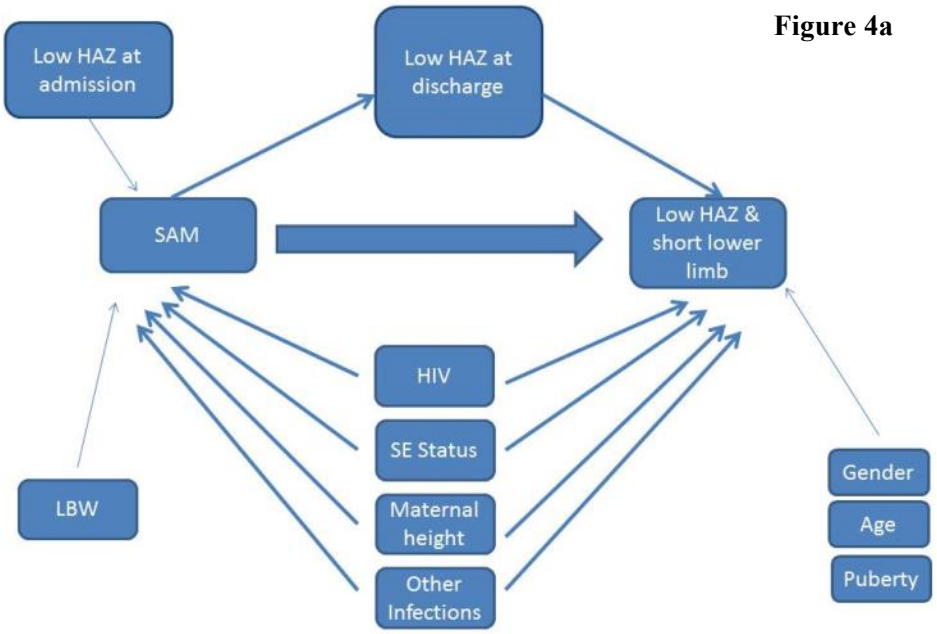


Figure 4a

Dyslipidaemia

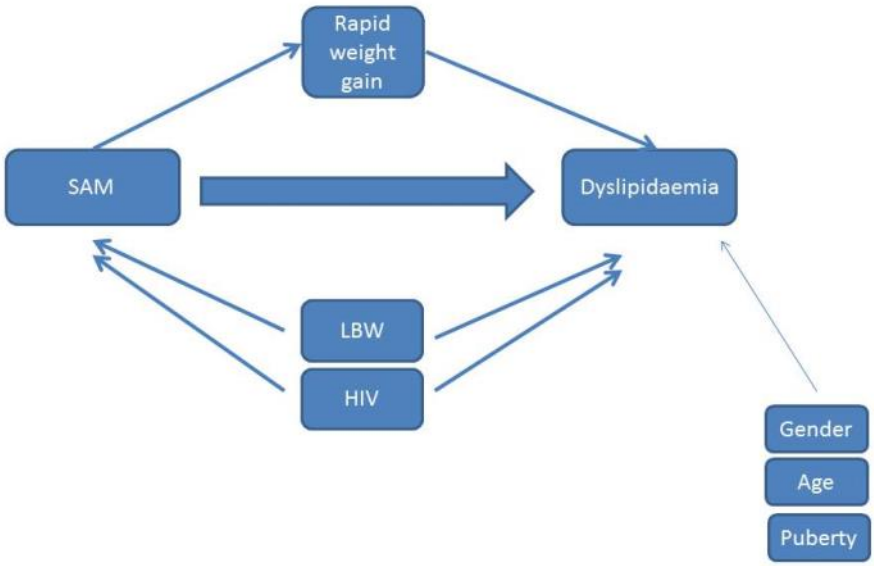


Figure 4b

Figure 4c

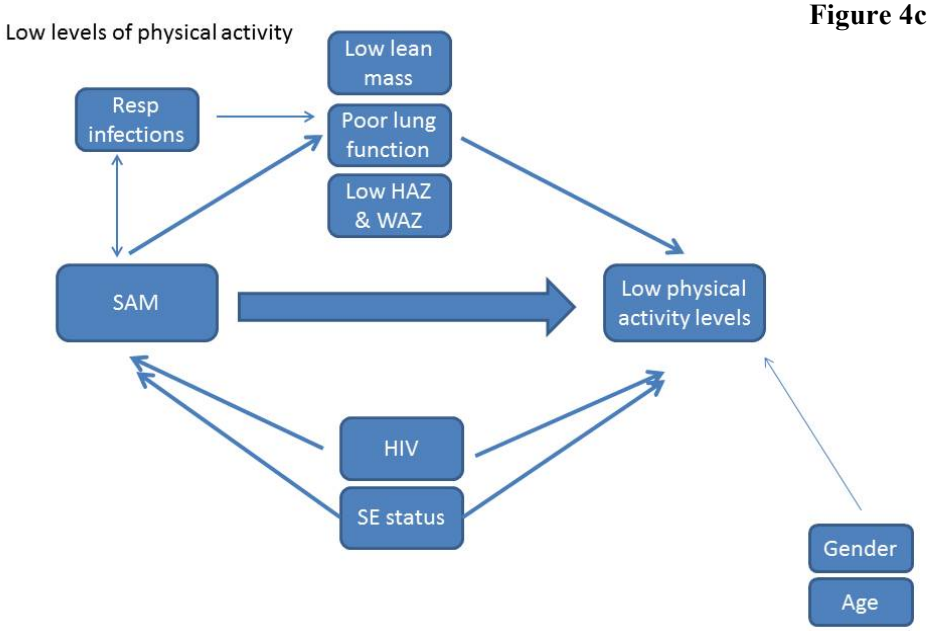


Figure 4d

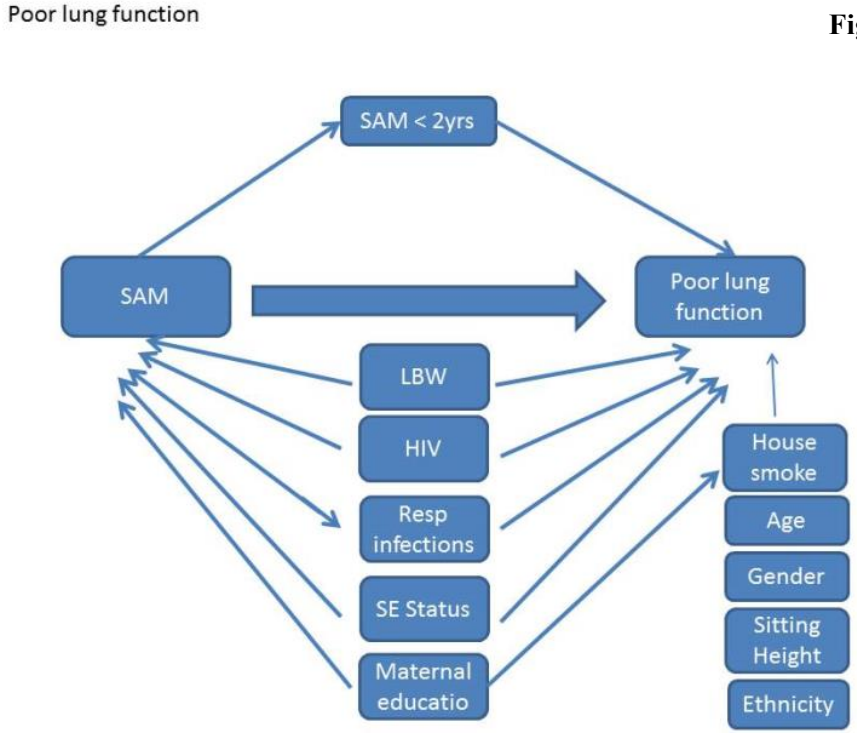


Figure 4: Conceptual frameworks for each of the four hypotheses in this study linking SAM with outcomes 7 years later including potential confounders; (4a) SAM leads to stunting, particularly shorter legs (4b) SAM leads to dyslipidemia (4c) SAM leads to lower levels of physical activity (4d) SAM leads to poor long-term lung function.

2.7.2 Linear Regression Analysis

Linear regression is a statistical technique for modelling the relationship between a dependant variable (y) and one or more explanatory variables (x), with the assumption that it is linear. It can be used to predict variable y based on x, or to identify and quantify the relationship between y and x. In this study, I largely use multiple linear regression analysis to identify and quantify the relationship between an outcome such as stunting and the explanatory variable "study group" (i.e. case/sibling/community control), as well as including potential confounders in the model as additional explanatory variables. Linear regression analysis requires the dependant y variable to be normally distributed, which is the case for most of the outcomes of interest. Normal distribution was determined using a skewedness value between -1 and 1. For skewed outcomes, the variable is transformed into a log-normal distribution for analysis. All analyses for this study were conducted using Stata12 software (StataCorp LP, USA).

As cases and controls were matched in certain variables at recruitment, "matched" analysis was considered. There is an argument that matched case-control studies should always be analysed in a matched format, however if there is no problem of *sparse* data, unconditional analysis is a better option as it often improves precision and avoids the need to exclude unmatched cases[201]. This study is not a case-control study as that would mean recruiting at the point of the outcome (e.g. stunting) and analysing retrospective risk factors (i.e. did they have SAM in the past or not?). This is a prospective cohort study with the addition of "unexposed" control groups hence matching of cases and those in the "unexposed" groups was primarily to reduce the range and breadth of potential confounders rather than with the intention of conducting matched analysis[202].

2.7.3 Principle Component Analysis (PCA)

I used principle component analysis (PCA) to create wealth scores based on asset data. PCA is a multivariate statistical technique that allows linear transformation of many variables into fewer, uncorrelated variables which represent the majority of the information in the original data set[203, 204]. It is commonly used for transforming asset data collected via DHS variables into an asset score which is indicative of socio-economic status [205]. This score cannot be used to compare one population with another, unless the exact same variables are used, however it is useful in this study for comparing socio-economic status between the study groups and adjusting for intra-sample variability in SES. Although PCA analysis gives us an idea of wealth at a household level, it does not allow us to differentiate how resources are allocated within the household. The variables included in my PCA for creation of a wealth score, which was then cut into quintiles, are listed in **Table 4** below. Quintile 1 has the lowest socio-economic status and quintile 5 the highest. As wealth and stunting are closely associated in almost all societies, a graph plotting the relationship between these two factors was used to test the accuracy of PCA-created wealth quintiles (**Appendix 10**)[206, 207].

Questions included in PCA analysis
In the dwelling is there electricity?
In the dwelling is there a bicycle?
In the dwelling, is there a motorbike?
In the dwelling, is there a car?
In the dwelling, is there a koloboyi? (basic lamp)
In the dwelling, is there a paraffin lamp?
In the dwelling, is there an oxcart?
In the dwelling, is there a TV?
In the dwelling, is there a mobile phone?
In the dwelling, is there a telephone (landline)?
In the dwelling, is there a bed with a mattress?
In the dwelling, is there a sofa?
In the dwelling, is there a table and chair?
In the dwelling, is there a refrigerator?
Do any members on the household own a watch?
Do members of the household work their own agricultural land?
Do any members of your household have a bank account?
What is the principle household source of drinking water?
What is the principle type of roofing in your dwelling?
What is the principle type of toilet facility used by the household members?
What is the principle type of flooring in your dwelling?

Table 4: Outcomes used in Principle Component Analysis (PCA) to generate wealth score

3 Chapter 3: Cohort Profile

This chapter is the first of the results chapters but it simply describes the sample and basic demographics of the study groups, including results of recruitment, mortality rate, HIV prevalence and a brief exploration of the outcomes for the original study groups in the synbiotic trial. It also describes missing data, which is largely due to loss to follow up, and presents results of questionnaire validity checks. The results in this chapter should give a general background profile to the study groups, before moving on to the next chapters which give results specific to growth, body composition, physical function and lung function.

3.1 Sample Description

I recruited 320 living case children in the study. The recruitment flow diagram (**Figure 5**) shows numbers recruited, died and lost to follow-up for case children, starting with the original number of admissions during PRONUT study up to current follow up. A sibling control was sought for every case child recruited, however not all cases had siblings, some didn't have any siblings within the eligible age range (4-15 years), and some had siblings who were not available or declined to take part, hence there is a slight disparity between number of cases and number of siblings. A community control was sought for every recruited case child; number of families who had an eligible control child but declined to take part was not recorded. Some case children live in very rural communities where locating a community control family that would consent was difficult. Many potential community control families had no experience of study participation and no history linking them to the NRU at QECH, hence they were more difficult to recruit than case families. Following recruitment, 11 sibling controls and 11 community controls were excluded from analysis as they had been admitted for SAM in their lifetime. 217 sibling controls and 184 community controls are included in the final analyses.

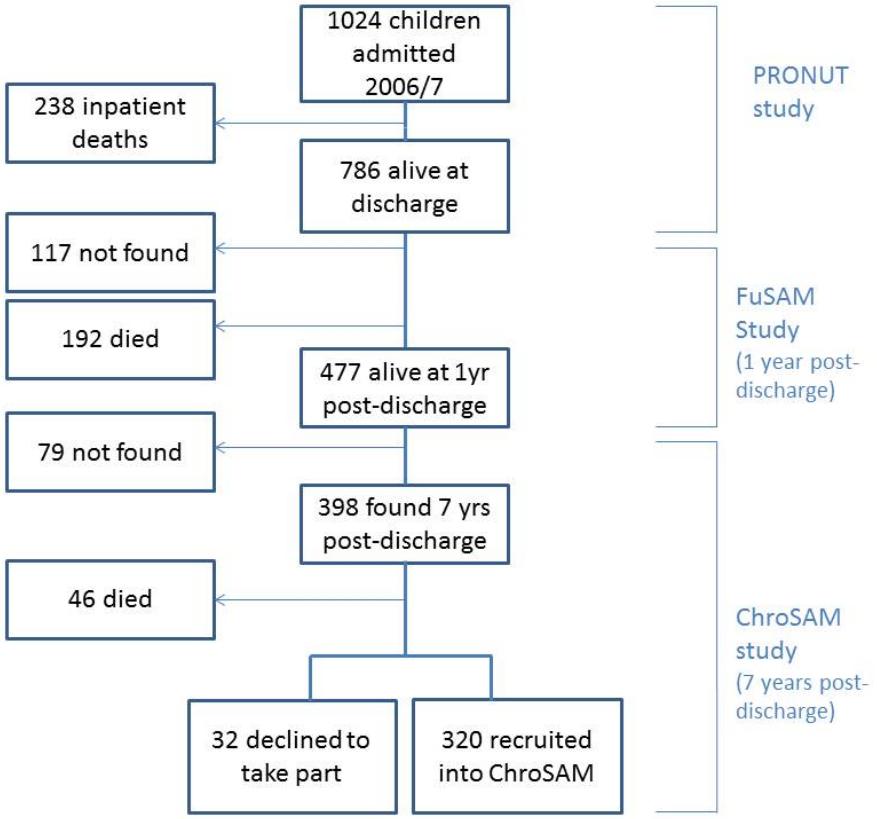


Figure 5: Recruitment flow diagram for case children in the cohort, starting with original recruitment in 2006 (PRONUT)[88], followed by 1-year follow up (FuSAM)[97], and present follow up (ChroSAM).

The number of children with a valid measure for analysis varies across all the outcomes. Carers and participants had the option to consent to some outcome measures but not others, some measures are effort-dependent (spirometry and physical function), and some variables required more cleaning of implausible results than others. Details of the number of readings removed due to data cleaning can be found in each of the relevant results chapters. Spirometry results have a particularly complex cleaning process, hence a specific flow diagram for spirometry outcomes can be found in **Chapter 7**. A summary of the number of participants included in analysis for all of the main outcomes can be seen in **Table 5**.

	Cases	Sibling Controls	Community Controls	Total children
Recruited (reported health)	320 (309 well, 11 sick)	217 (215 well, 2 sick)	184 (182 well, 2 sick)	**721**
HAZ	299	203	159	**661**
WAZ	215	94	117	**426**
Sitting height	292	198	151	**640**
MUAC	302	204	159	**664**
Head circumference	302	203	159	**664**
Waist circumference	301	203	157	**661**
BIA	295	197	151	**643**
Skinfold thicknesses	280	187	141	**608**
Handgrip strength	298	199	154	**651**
iStep exercise test	222	149	126	**497**
Accelerometer data	44	36	41	**121**
Spirometry FEV1Z (failed traces)	199 (+38 fails)	142 (+22 fails)	121 (+10 fails)	**462**
Spirometry FVCZ (failed traces)	186 (+51 fails)	138 (+26 fails)	115 (+16 fails)	**439**

Table 5: Number of participants included in analysis for each of the main outcomes

3.1.1 Missing data

It is important to consider whether missing data could be biasing study results. The main reasons for missing data in this study are children who died before follow-up, and children who could not be located. Minor reasons for missing data specific to each outcome are discussed in the relevant results chapters. Missing data due to deaths in the cohort group could result in survivor bias, discussed below. Most of the potential bias resulting from missing data cannot be identified however it is possible to explore whether there are common factors among the missing data which could suggest that their absence may be biasing results. Deaths and loss to follow-up during PRONUT and FuSAM studies have already been explored[87].

Some variables of those lost to follow up are available from baseline (PRONUT) study. When comparing those included (n=320) and those lost to follow up over the whole study period (n=190), I found that those lost to follow-up are significantly younger, less likely to be orphans, less likely to have parents who have divorced, less likely to have HIV positive mothers and less likely to have mothers or fathers with no education. These results suggest that those lost to follow up might be more affluent than those included. See numbers in **Table 6**. Although urban/rural

status was not collected, it was noted by the study team that those in rural areas were easier to locate than those in more densely populated urban areas as urban dwellers are less likely to know their neighbours and more likely to move often due to rent prices. It is possible that those lost to follow up are slightly more affluent and more urban.

3.1.2 Other Bias

As 46% (476/1024) of the original cohorts are known to have died, **survivor bias** is an important consideration for this study. If those who have died have systematic differences to those who survived, our case group could be misrepresenting the long-term effects of SAM. Kerac previously explained that those who died between admission and 1 year post-discharge were more likely to have marasmus than kwashiorkor, were more wasted and stunted on admission, were more likely to be HIV positive and were more likely to have a disability[87]. Cases who have died since the 1-year follow up (n=46) are more likely to be HIV positive and previously contracted TB than those in the survivors' case group. They are also more likely to have HIV positive mothers, mothers who have died, mothers without secondary education, and shorter and thinner mothers. This suggests survivor bias may be masking some of the long-term effects of SAM as the most malnourished, least healthy and those with the poorest maternal health have not survived.

Selection bias could also be affecting our community control group as it is possible that those who chose to participate differ systematically from those who declined. I cannot assess this as I do not have data on those who declined but I can speculate that they may be different in SES or health status; it may be that those who chose to participate have had some experience of hospital care and were therefore less wary than those who are healthy and therefore never had dealings with the QECH.

Age is an important variable, particularly when calculating anthropometric z scores. Recall error is likely to be affecting reported age of the participants; however this only constitutes **recall bias** if it is systematic in one direction or differentially affects one study group over another. As age was reported both at admission and during this follow-up for the case group, I could compare the reported ages for systematic error. 28/320 cases had a reported age that was more than 6 months different to the original reported age; 17 had increased in age since admission and 11 had decreased. Although the original age reported at admission is likely to be more accurate as it is temporally closer to the child's date of birth, I ran all analysis with the ChroSAM reported ages as any recall error affecting these is likely to equally effect the control's reported ages as well.

Lastly, due to the data collectors not being blinded to the case/control status of the participants, **observer bias** could be affecting the results. The data collectors may have unconsciously influenced the participants or unconsciously alter measurements, differentially for cases and

controls. This is unlikely to have affected anthropometric outcomes as 2 operators had to take independent measures; it is also not very relevant for BIA readings which are largely objective and also accelerometer data where operators were not present during measurement. Observer bias could be relevant for hand grip strength, iStep test and spirometry where varying levels of encouragement or subtle subconscious differences in behaviour towards the case children could affect their performance differentially to controls.

3.1.3 Study group Demographic and Health Characteristics

The background characteristics of the cases, sibling controls and community controls are presented in **Table 6**. The sex ratio and birth order are similar across the groups; the ages of cases and community controls are similar but sibling controls are older on average. Regarding clinical history, more cases are HIV positive and have a history of TB than controls, and cases are more likely to have visited a health clinic in the past 6 months. Regarding other hospital admissions beside SAM, this is actually slightly higher for community controls than cases. Perhaps this reflects the type of community control recruited i.e. those more likely to seek medical care or have medical issues, or perhaps it just reflects the high rates of morbidity among all children in the community.

Education variables are not significantly different across the groups, but cases and siblings come from more disadvantaged families than community controls. Cases are more likely to be orphans or come from single parent families than community controls and have mothers with smaller MUAC and lower BMI. Mothers of SAM survivors are not more stunted than other mothers in the community, as might be expected. There is no significant difference in prevalence of poor maternal mental health between the cases and controls, but community controls are more likely to live in families subject to domestic violence than case children.

Reported home environment is similar across the groups, but cases come from slightly lower socioeconomic background than community controls. Smoking during pregnancy is a risk factor for poor growth and greater NCD risk signs in children, however reported foetal tobacco exposure in this sample was very low, with less than 1% (4/526) of mothers smoking during pregnancy and no significant difference between case and control groups[208].

	Cases (n=320)	ChroSAM deaths (n=46)	Sibling controls (n=217)	Community controls (n=184)	Cases Lost to Follow Up (190)
Basic Demographics					
Median age /would be age (years)	9.3 (range 7.4 – 20.1)	10 (range 8.1 – 16)	10.9 (range 4 – 15.6)	9.1 (range 5.1 – 15.1)	8 (range 7 - 19)
Males (gender)	174 (55%)	28 (57%)	106 (49%)	96 (52%)	108 (57%)
Median Birth Order	2 (IQR 1-4)	3 (IQR 1-9)	2 (IQR 2-3)	2 (IQR 1-3)	2 (IQR 1-3)
Clinical History					
HIV					
Seropositive	90/320 (28%)	25/46(53%)	9/217 (4%)	5/184 (3%)	44/190(23%)
Seronegative	208/320(65%)	20/46 (43%)	130/217(60%)	95/184 (52%)	121/190(64%)
Unknown	22/320 (7%)	1/46 (2%)	78/217 (36%)	84/184 (46%)	25/190 (13%)
History of TB	18/317 (5.7%)	4/38 (11%)	1/215 (0.5%)	1/179 (0.6%)	4/173 (2%)
History of pneumonia admissions	10/317 (3%)	///	15/213 (7%)	9/180 (5%)	///
Ever admitted to hospital (except SAM)	70/318 (22%)	///	53/213 (25%)	54/180 (30%)	///
Visited outpatient clinic in past 6 months	115/315 (37%)	///	56/214 (26%)	51/179 (28%)	///
Education					
School grade achieved (median)	2 (IQR 2-3)	///	3 (IQR 1-5)	3 (IQR 2-4)	///
Didn't attended school this year(>5.9yrs)	21/315 (7%)	///	18/196 (9%)	9/176 (5%)	///
Receive school meals	206/313 (66%)	///	///	120/173 (69%)	///
Family					
Mother died	51/303 (17%)	11/30 (37%)	///	4/160 (3%)	8/159 (5%)
Father died	59/286 (21%)		///	9/151 (6%)	
Orphaned	34/334 (10%)	6/31 (19%)	///	2/160 (1%)	0/190 (0%)
Parents separated	141/280 (50%)	7/14 (50%)	///	59/172 (34%)	25/139(18%)
Mother HIV positive	83/222 (37%)	10/20 (50%)	///	23/135 (17%)	23/81 (28%)
Mother's age (median)	32 (range 18-62)	34 (range 28-47)	///	30 (range 19-50	///
Maternal Height cm (mean) (SD)	156.9 (5.7)	155.5 (3.1)	///	156.4 (5.9)	///

Maternal MUAC mm (mean) (SD)	271 (39)	260 (21)	///	282 (41)	///
Mother's BMI (mean) (SD)	22.9 (4.1)	21.8 (2.3)	///	23.6 (4.3)	///
Parity~ (median)	3 (IQR 3-5)	3 (2-4)	///	4 (IQR 3-5)	///
Proportion of siblings died (mean) (SD)	0.09 (0.2)	0.4 (0.3)	///	0.04 (0.1)	///
Mothers victims of domestic violence	58/257 (23%)	0/9 (0%)	///	46/135 (34%)	///
Poor maternal mental health¥	73/275 (27%)	5/15 (33%)	///	52/155 (34%)	///
Mother with no social support	13/288 (5%)	0/11 (0%)	///	5/173 (3%)	///
Home environment					
Exposed to tobacco smoke	47/317 (15%)	5/35 (14%)	///	30/174 (17%)	///
Cooking with solid fuel in the home *	47/317 (15%)	2/35 (6%)	///	21/174 (12%)	///
Unimproved toilet Φ	244/315 (77%)	20/24 (83%)	///	140/173 (81%)	///
Socioeconomic status					
Mother education					
None	56/309 (18%)	5/14 (35%)	///	22/173 (13%)	13/155 (8%)
Primary	121/309(39%)	6/14 (43%)	///	65/173 (38%)	108/155(70%)
Secondary	132/309(43%)	3/14 (21%)	///	86/173 (50%)	34/155 (22%)
Father with no education	39/270 (14%)	3/13 (23%)	///	11/150 (7%)	5/122 (4%)
Father unemployed	120/255(47%)	6/9 (67%)	///	62/158 (39%)	///
Lowest wealth asset quintile	67/311 (22%)	3/24 (13%)	48/215 (22%)	30/174 (17%)	///

Table 6: Characteristics of cases recruited, cases that have died since 1 year follow-up, sibling controls, community controls and cases lost to follow up. ~ parity defined as number of children in family alive; ¥ poor maternal mental health defined as SRQ score ≥7; * cooking smoke defined as cooking with wood or charcoal inside the home; Φ unsanitary toilet defined as open pit latrine or field or bush. /// indicates no data, largely because family characteristics for siblings were presumed to be the same as cases

3.1.4 HIV

As HIV is likely to have a large impact on all study outcomes, HIV status in the sample warrants further description. **Figure 6** shows the proportion of HIV positive, negative and unknown status children in each of the study groups and in those who died since 1 year post-discharge. The proportion of HIV positive children in the case group is considerably larger than that in the sibling and community control groups, and the proportion with unknown status is considerable in the control groups as many families declined to be tested. Of the HIV positive children, 76/104 (70%) are taking both Cotrimoxazole (COT) and Antiretroviral medication (ARVs). 11/104 (11%) have taken COT but not ARVs, and 6/104 (6%) have never taken COT or ARVs. Of those taking ARVs, only 50/76 (66%) report not having missed any doses of ARV in the past 3 months. A small number of families reported problems taking COT (5/87) or problems taking ARVs (6/76); reasons for these problems included the child being too sick to go to collect the medication, the mother being too busy to collect the medication or the medication being out of stock. Particularly for ARVs, problems also included the child refusing the medication, the medication tasting bad and/or being difficult to swallow and there being no food to take medication with.

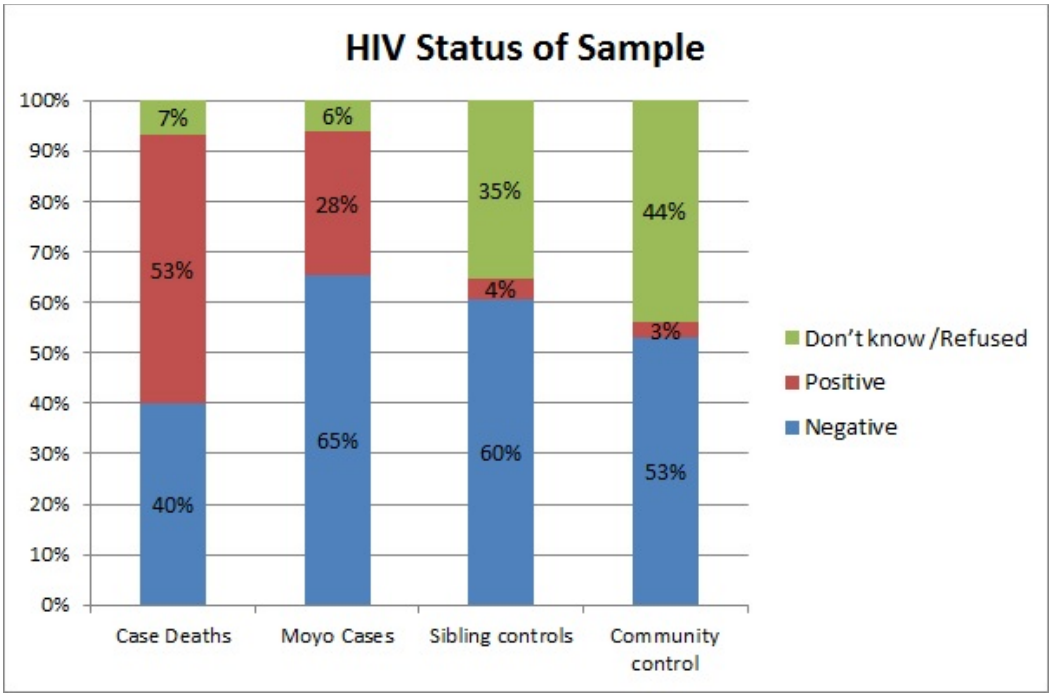


Figure 6: Graph showing proportion HIV positive, negative and unknown status children in each of the study groups as well as in those who have died since 1 year post-discharge.

3.2 Mortality

This section explores the mortality of the case group across the different follow-up periods. I am describing mortality here rather than in the main results section as it is very difficult to conclude whether the mortality rate found is higher or lower than the normal population rate without a control group; only rough estimates can be made.

Mortality between the 1 year follow-up (FuSAM) and the present follow-up was not expected to be high as mortality rates after 2 years of age generally drop compared to younger children, and because so many of the cohort had already died, suggesting that those that remained were the healthier ones. Since admission and 1-year post-discharge, a total of 42% (427/1024) of the cohort had died. Mortality was highest during initial inpatient treatment where 238 of the 427 deaths occurred (56%). Of the 427 deaths, 62% were HIV positive and 17% HIV negative, as previously described [97]. After the high mortality on the ward, the death rate has continued to decrease with increased time since discharge. Of the 398 children who were found during this 7 year follow-up, 46 had died since FuSAM (12%). Of the 46 deaths, 25 (51%) were HIV positive, 10 (22%) had a disability, and 11 (24%) had neither HIV nor a disability; **Appendix 11** shows pie chart of all known causes of ChroSAM deaths. **Figures 7a and 7b** show Kaplan Meier Time-to-death curves; they are extended versions of the curves published after the 1-year follow up[97]. The tables below the curves show numbers at risk at the beginning of each period. With this denominator change, the y-axis is mortality *probability* rather than percentage. The steep initial slope of the curve highlights that probability of death was highest within 1 month of admission, and continued to rise until 2 years post-admission. Time-to-death curves by HIV status (**Figure 7b**) highlight the significant adverse impact of HIV; deaths in the HIV negative patients slow significantly soon after admission but continued to rise more sharply for those in the HIV positive group, even as long as 8 years post-admission. The likely explanation for the step up in deaths around 90 months post-admission in recall bias with regard to date of death.

The difficulty in analysing mortality is assessing whether those children who died between 1 and 7 years post-discharge are excess mortality over and above background rates, or whether statistically some were going to die anyway. Without following a control group, I can only refer to national mortality rates in order to try to estimate this. WHO life tables for Malawi in 2012 show mortality rate in children aged 1 to 4 years is 0.007, and aged 5 to 9 years is 0.003[209]. These are age-specific death rates (nMx) where:

x: exact age at beginning of interval
M: number of deaths
n: width of the interval (in years)

Age-specific death rate is calculated as:

$$\frac{\text{The number of deaths in a year to persons between exact ages x and x+n}}{\text{The number of person years lived by the population between exact x and x+n in the same year}}$$

By focussing on specific years, for example 2011, I calculated how old all the case children would be in 2011 (removing any who died before that year) and then calculated the person years for all those who were between 5 and 9 years in 2011. Then I divided the number of deaths between ages of 5 and 9 in 2011 by the number of person years. However, due to small numbers, there is relatively large variation in the number of deaths each year; resulting in mortality rate between 0.05 and 0.002 from 2008 to 2013. Mean mortality rate across the period is 0.014, significantly higher than national rate of 0.003 for that age group; the highest death rate is at the start of the period (i.e. 2 years post-discharge). Graph of mortality rate across ChroSAM follow-up years can be seen in **Appendix 12**.

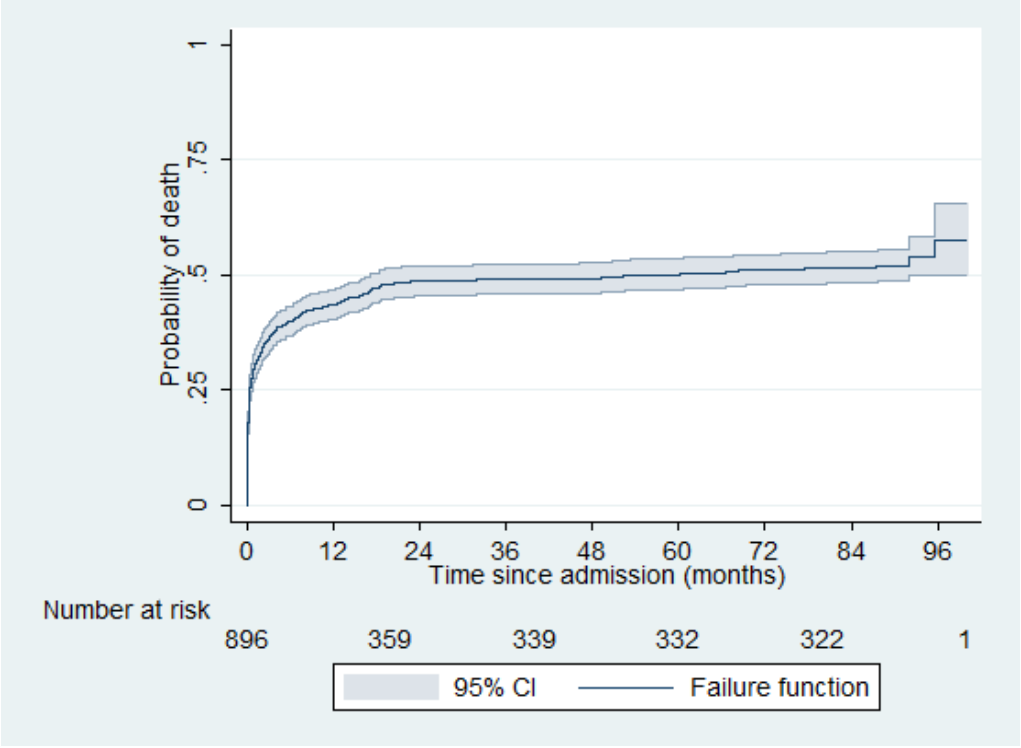


Figure 7a: Kaplan Meier time-to-death curve for whole sample since admission with SAM. Curves show probability of death, table below graph shows number of children at risk across each period

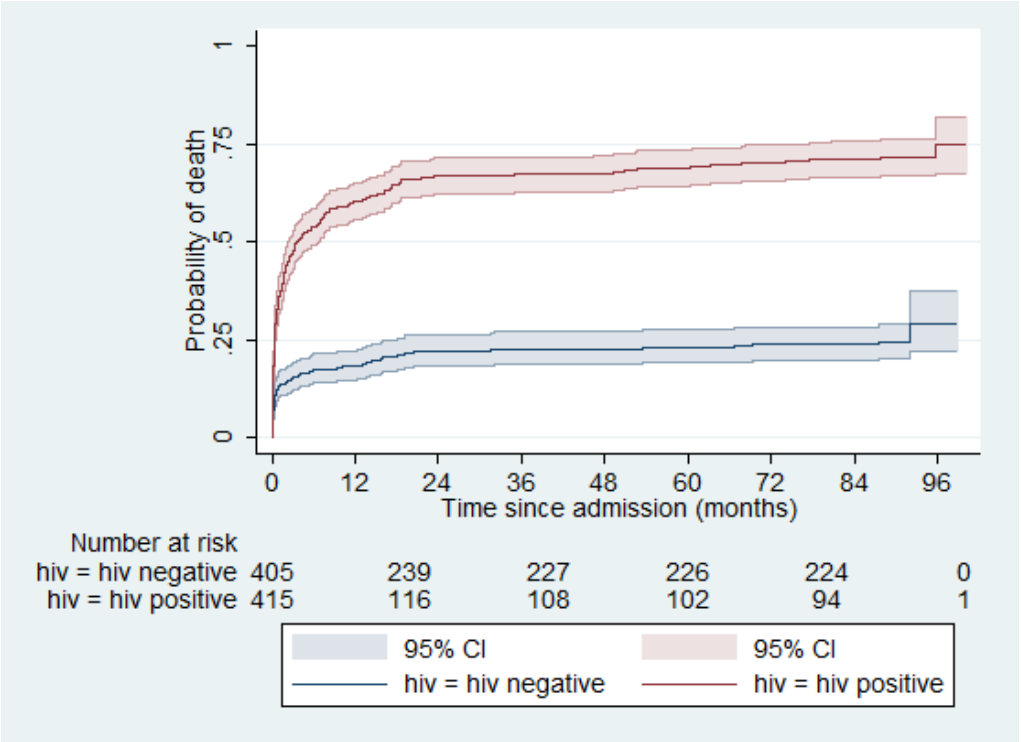


Figure 7b: Kaplan Meier time-to-death curves by HIV status since admission with SAM. Curves show probability of death, table below graph shows number of children at risk across each period

3.3 Original Synbiotic/Control groups

The original cohort arose from a randomised control trial testing the benefits of providing synbiotics to SAM suffers during treatment (PRONUT study)[88]; the probiotics were found to have no effect and therefore the synbiotic group and control group were combined to form this cohort. It is prudent to check whether the original synbiotic group remains different to the original control group. I explored differences in mortality and nutritional status for the two original groupings within the SAM survivors. Time-to-death curves in **Figure 8** show that there is still no significant difference in survival between synbiotic and original control group. Equally there is no significant difference in HAZ, WAZ and BAZ at 7 years post-discharge between synbiotic and original control groups; results in **Table 7**.

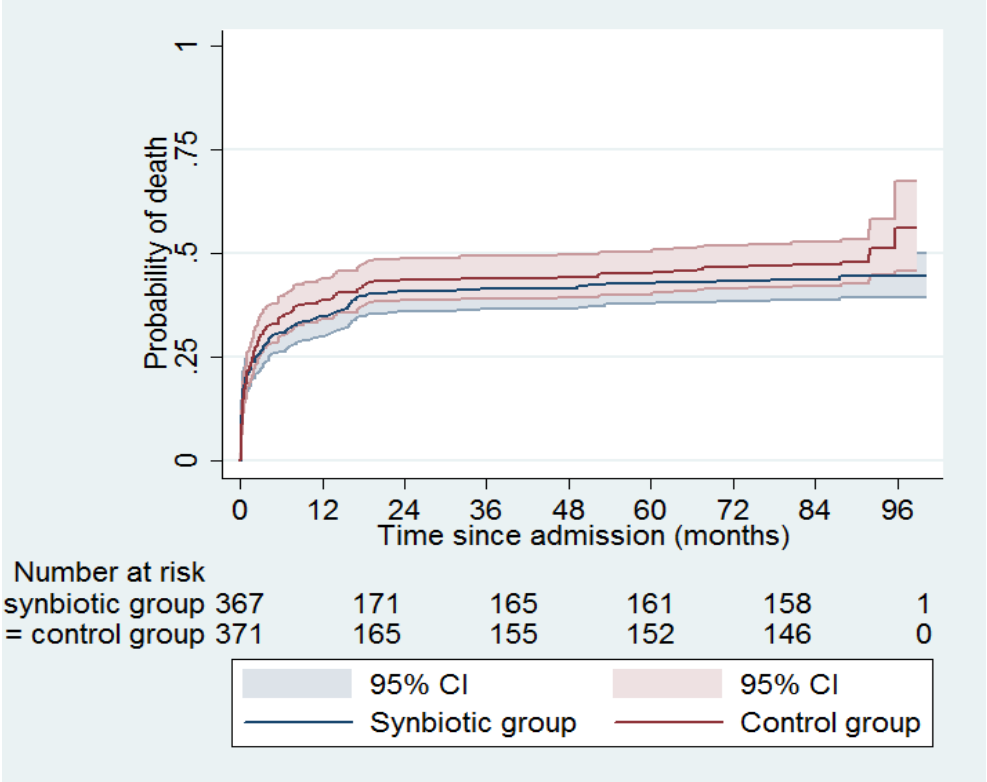


Figure 8: Kaplan Meier time-to-death curves by original synbiotic/control status

	Original control group n=131	synbiotic group n=148		
	Mean (SD)	Mean (SD)	Unadjusted difference (95% CI)	Adjusted difference (95% CI)
WAZ	-1.44 (0.9)	-1.63 (1.0)	-0.19 (-0.5, 0.1)	-0.18 (-0.4, 0.1)
HAZ	-1.77 (1.1)	-1.84 (1.3)	-0.07 (-0.4,0.2)	-0.06 (-0.3, 0.2)
BAZ	-0.85 (0.9)	-0.86 (1.0)	-0.01 (-0.2, 0.2)	-0.04 (-0.3, 0.2)

Table 7: Means and differences for WAZ, HAZ and BAZ for the original controls and case groups in the PRONUT study. Adjusted differences include HIV status and SES in the regression model. No differences have a p value <0.05.

3.4 Questionnaire Repeatability

Due to constant time pressures and unique requirements of each and every family, repeat questionnaires were only completed for 14 participants. I used Cohen's kappa, a measurement of agreement between repeat measures, to assess questionnaire repeatability[210]. Means, kappa and kappa interpretation are presented in **Table 8** for a range of variables. Kappa is the amount by which agreement exceeds chance, divided by the maximum possible amount by which agreement could exceed change. Perfect agreement is kappa=1, no agreement is kappa=0. Landis and Koch (1977) set cut-off values for kappa in order to define agreement as poor, fair, moderate, good and very good[211]. Sex, birth order, history of TB, HIV status, school achievement and sight impairments all had perfect or very good agreement between first and second questionnaires. Mother's and father's occupation had relatively low agreement, likely reflecting a poorly designed question with overlapping response options, for example a mother can be both "unemployed" and a "housewife". Questions about children's behaviour in the Disability screening questionnaire only had fair or moderate agreement.

Variable	Mean (SD) 1st time	Mean (SD) 2nd time	Kappa (k)	Kappa interpretation
Sex	1.36 (0.5)	1.36 (0.5)	1.00	Perfect agreement
Birth order	2.21 (1.5)	2.29 (1.5)	0.90	Very good agreement
Started puberty?	1.92 (0.3)	1.86 (0.4)	0.63	Good agreement
Number of outpatient visits in past 6 months	1.36 (2.8)	0.43 (0.8)	0.44	Moderate agreement
History of TB?	2 (0)	2 (0)	1.00	Perfect agreement
HIV status	1.6 (0.5)	1.3 (0.5)	1.00	Perfect agreement
Highest level of school achievement	3.43 (2.6)	3.36 (2.6)	0.91	Very good agreement
Ever experienced domestic violence?	1.4 (0.5)	1.6 (0.5)	0.67	Good agreement
Fathers occupation	2 (1.5)	1.5 (0.8)	0.23	Fair agreement
Mothers occupation	3.33 (3.0)	4.67 (3.5)	0.10	Poor agreement
Does child have difficulty seeing?	1.1 (0.3)	1 (0)	0.91	Very good agreement
How much difficulty does child have completing a task?	1.4 (0.6)	1.3 (0.7)	0.43	Moderate agreement
How much difficulty does child have controlling their behaviour?	1.5 (0.8)	1.5 (0.9)	0.27	Fair agreement

Table 8: Results of questionnaire validity analysis, including means for each repeat, Cohen's kappa and kappa interpretation as published by Landis and Koch[211].

4 Chapter 4: Growth and Anthropometric Status

This chapter presents specific methods and results for the first of the four areas of outcome presented in this thesis: long term effects of SAM on growth. Following a description of the anthropometric methods, the results are presented starting with growth of the whole sample followed by the main results comparing growth outcomes of cases to controls. The longitudinal results for sibling controls and cases between 1 and 7 years post-discharge are then presented followed by an exploration of HIV and growth, factors at admission which predict poor growth outcomes and association between reported birth size and growth outcomes.

4.1 Chapter-specific Hypotheses

As mentioned in the Chapter 1: Background, growth is an important determinant of both short- and long-term health and quality of life[84, 96]. During the one year follow-up of this cohort, survivors were found to be significantly more stunted that their siblings[97], hence linear growth is a major outcome being considered during this current follow-up. Additionally, the "Thrifty Phenotype" hypothesis postulates that essential growth, such as head and core body, is preserved in the place of less-essential growth, such as limbs[40, 110]. Hence another area of exploration for this study is analysis of sitting height, leg length and head circumference, as evidence for "Thrifty Growth". Hypothesis one and two relate to the growth outcomes being explored in this chapter, and are restated below:

1. Compared to controls, SAM survivors have impaired linear growth, specifically
    a. Smaller HAZ
    b. Shorter leg length
    c. But preserved head circumference

2. Compared to sibling controls, linear growth velocity is continuing at normal rates resulting in no catch-up growth since 1 year post-discharge.

4.2 Specific Methods

4.2.1 Anthropometry

Weight: weight was measured using Marsden digital medical weighing scales (Marsden Weighing Group, UK). The children were measured without their shoes and any heavy clothing, standing in the centre of the scales with their hands by their sides. Measurements were recorded to the nearest 0.1kg.

Height: to measure the child and parent's standing height, a Leicester Height Measure was used (Marsden Weighing Group, UK). Shoes and hair ornaments were removed and the participant stood straight with their feet flat on floor, their heels against the heel plate and their head horizontal in the Frankfurt plane (**Figure 9**). Prior to recording the measurement, the participant was asked to breathe in and then relax. The headboard was adjusted on expiration and measurement taken. The measurements were recorded to the nearest 0.1cm.

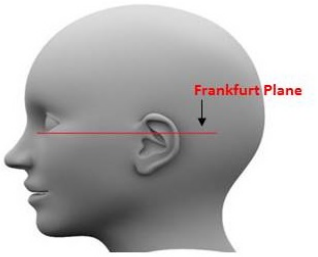


Figure 9: Image illustrating the Frankfurt plane which is used to position the head during height measures

Sitting height: The child was seated on the specially designed stool which accommodates the base of the Leicester Height Measure and has adjustable foot rests (**Image 4**). The child's spine and bottom were touching the stadiometer backboard and their feet were on the footrest so that their hips were at right angles and their thighs horizontal with the floor. As with standing height, the child was asked to breathe in and relax and the measurement was taken while the child exhales, recorded to the nearest 0.1cm.

Image 4: Specially-designed stool for measuring sitting height; the Leicester Height Measure base fits onto the top of the stool for sitting on and there are adjustable foot rests to ensure the child's thighs are horizontal with the floor

Head circumference: A *Rollfix* Hoechstmass measuring tape was used for all circumferences. Hair ornaments were removed so that any hair could be compressed close to the skull and the child stands with their head in the Frankfurt plane. The tape goes around the head so that it rests on the fore head just above the eyebrows, and at the widest point of the curve at the back of the head (occipital bone). The measurement was recorded to the nearest 0.1cm.

Image 5: Measuring head circumference at a participant's home *(written permission was obtained from subjects for all photographs featuring children in this thesis)*

Lower limb length: this was measured with the child siting on the 'sitting height stool' so that their feet were resting on the appropriate footrest in order to have their thighs perpendicular to the floor. Using the Leicester Height Measure, the feet were placed on the base of the stadiometer and the headboard bought down to rest on the child's right thigh, just above the knee. This measures the distance between the top surface of the thigh (above the condyles of the femur and about 4cm above the patella) and the bottom of the foot, as seen in **Figure 10**. The measurement was recorded to the nearest 0.1cm.

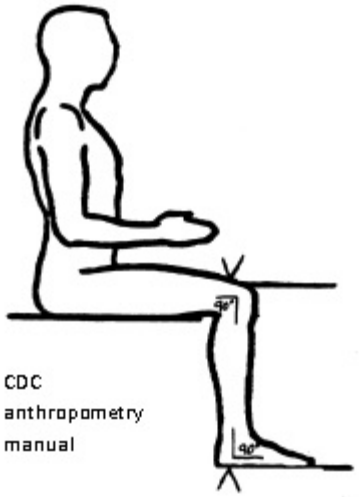


Figure 10: Diagram illustration distance measured for lower limb length, source: CDC anthropometry procedures manual[212]

Mid-upper arm circumference (MUAC): MUAC was measured using specifically designed 'MUAC tapes' produced by Teaching Aids at Low Cost (TALC). MUAC was measured on the left arm, while the arm was straight and relaxed, and the reading recorded to the nearest 1mm. The midpoint of the upper arm was located by measuring the distance the acromion process and the olecranon process, while the arm was held across the stomach with the elbow bent at 90˚.

Image 6: Measuring MUAC of a participant at their home using a TALC MUAC tape.

4.2.2 Sample Size Calculations

As already mentioned, the sample size available for this study was constrained by the number of children available from the initial cohort recruitment. Based on actual sample size and using evidence from previous studies, I calculated power for the primary outcomes. Statistical power is the likelihood of detecting an effect if one does exist; greater power reduces the chance of erroneously accepting the null hypothesis when it is actually false (type II error). Sample size was calculated using Stata "*sampsi*" command.

The 1-year FuSAM study found a difference in WAZ of 0.55 and HAZ 1.13 between cases and sibling controls[97]. With our sample size for WAZ being 215 cases and 94 siblings, based on the difference seen in the FuSAM study, the comparison of the means is powered to 95% with significance level at 5%. Our HAZ sample size is also sufficiently powered based on FuSAM findings; comparing 299 cases and 213 siblings is powered to 100% at 5% significance level. For a more conservative estimate, I used the results of a meta-analysis by Richard et al. comparing HAZ of children with an incidence of wasting to those without; they found a range of differences

in HAZ, particularly depending on the age of the wasting insult[85]. The mean difference they found was 0.75 z scores, which is still detectable at 100% power with our sample size.

4.3 Analysis

Following brief presentation of the growth for the sample as a whole compared to the global norm, the **Primary analysis** is presented which firstly compared sibling and community controls to cases using regression analysis for:

- Weight
- Height (including sitting height and leg length)
- Nutritional z scores (WAZ, HAZ & BAZ)
- Mid-upper arm circumference (MUAC)
- Head circumference

These are all continuous outcomes hence simple and multivariable linear regression analysis was used to compare cases and both types of control. WAZ, HAZ and BAZ are the 3 standard indicators of nutritional status currently recommended by the WHO, each indicating different elements of nutrition and each with its own limitation. Potential confounding factors were adjusted for in multivariable linear regression; namely HIV status, age, sex, puberty and SES, based on *a priori* assumptions. A conceptual framework of potential confounders is depicted in **Figure 4a**, Chapter 2. Age and sex are included in multivariable regression analysis of z score outcomes as these z scores are generated from an external reference and scores are known to change across ages and sex.

Children with disabilities were considered for exclusion from analysis as some disabilities, such as cerebral palsy, can have large impacts on growth. However I decided to keep these children in the analysis (where accurate measures could be taken) in order to make the results of this study as generalizable to "SAM survivors" as possible. Much SAM does coincide with disability; hence it is important to consider the long-term outcomes of all children in the group.

Analysis of longitudinal WAZ, HAZ and BAZ data for case children and sibling controls also forms the second part of the primary analysis. Basic anthropometry of all siblings was measured during FuSAM study; by matching birth order I was able to link FuSAM sibling anthropometry to the sibling control selected in this follow-up. Some data is missing hence sample size for siblings with longitudinal data is 69. Change in nutritional z scores between 1 year and 7 years post-discharge are calculated for case children only and repeated measures ANOVA was used to compare whether z score changes of the cases is significantly different to z score changes of the sibling controls. As with any ANOVA, repeated measures ANOVA tests the equality of means; it

is used when members of a random sample are measured under a number of different conditions, in this case the WAZ, HAZ and BAZ are measured at different follow-up points.

Secondary analysis considered the impact of HIV and birth weight on growth as well as factors at admission which might predict poor growth at 7-years post-discharge, namely severity of stunting and wasting at admission, presence of oedema, time taken to recover and age at admission for case children only. Using linear regression, cases with different admission characteristics were compared to each other and in addition the two groups of cases were also compared to the control groups combined. Comparing the outcomes of the cases to each other internal is interesting but may not be relevant to the wider community; hence both types of comparison were conduct. For example, if the HAZ of those with oedema at admission and those without oedema at admission is compared, I may find that one group are significantly more stunted than the other. However if I then compare these two groups to the controls, I may find that in fact both groups are still more stunted than controls, so the applicable difference of oedema at admission compared to other children is minimal.

4.3.1 Data Cleaning & Z scores

In order to check for erroneous data among the anthropometric measures, I plotted scatter graphs of each variable against age and height, and checked each of the outliers for notes on their paper files. Some files had notes confirming or correcting readings, for example noting if a child was particularly stunted. Highly improbable readings without explanation were removed from analysis. Following this, WHO *Anthro Plus* software was used to calculate weight-for-age (WAZ), height-for-age (HAZ) and BMI-for-age (BAZ) z scores, based on the WHO 2007 Growth Reference for 5-19 year olds (http://www.who.int/growthref/en/). The WHO reference was created using Box-Cox power exponential methods on data from 22 countries[213]. These references replaced the 1977 National Centre for Health Statistics (NCHS) growth references. As the WHO reference was yet to be released, the previous studies of this cohort, PRONUT and FuSAM, reported results using the NCHS reference. I have recalculated the z scores using admission, discharge and follow-up anthropometry into WHO z-scores in order to compare growth across the whole timespan of the cohort. *Anthro Plus* uses default values (**Table 9**) to flag extreme z scores which may be erroneous. I used these flags to identify further anomalous values, and again checked the paper files for notes. Those readings outside of the range in the table, without explanation, were removed. Some values beyond this range were allowed to remain in analysis as they were verified to be correct. The number of values which needed to be removed was low; 3 readings of height were excluded and sitting height had 5 values excluded. **Figure 11** shows two scatter plots for height against weight, before and after data cleaning; outlying values were either corrected or removed.

Beside anthropometry, date of birth (DOB) is another variable which is fundamental to z score calculations. There is no way of checking whether DOB is correct for sibling and community control children, however a method of cross checking age and DOB was implemented during data collection to try minimise errors. For case children I was able to check that all dates of birth were feasible by comparing them against date of admission for malnutrition; no case children had a reported DOB post admission date. There were some discrepancies between DOB reported during PRONUT study and our follow up, as already discussed in Chapter 3, Section 3.1.2 Bias.

Indicator	Lower SD	Upper SD
WAZ	-6	+5
HAZ	-6	+6
BAZ	-5	+5

Table 9: WHO standards for flag of extreme z score results

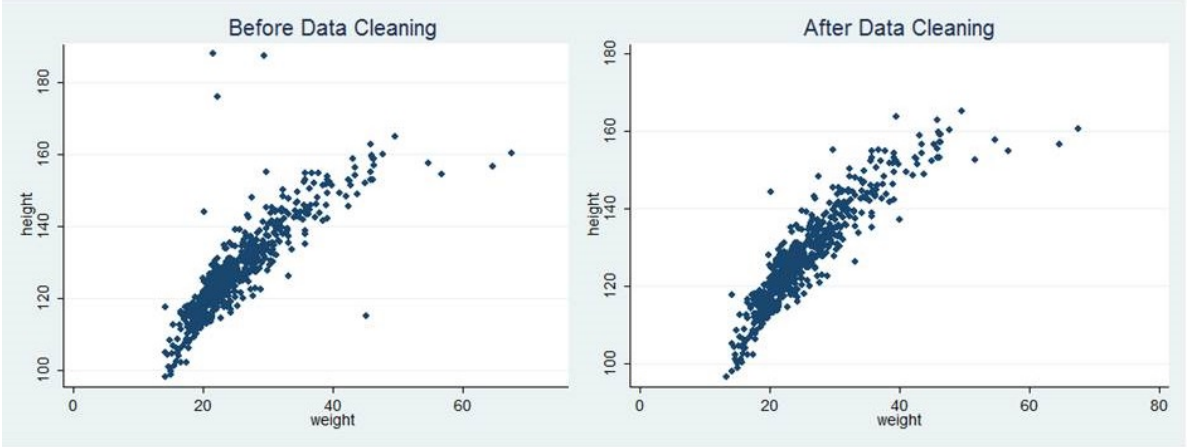


Figure 11: Scatter plots of height against weight; left hand plot is before data cleaning, right hand plot is after data cleaning. Notably some anomalies have been corrected or removed during cleaning.

4.3.2 Calculations and Statistics

Much of the analysis for growth was not of raw anthropometry but rather ratios and relative measures. Z scores were used to normalise the data, wherever reference tables were available, as described above. Besides z-scores, the following calculations were also applied to anthropometric growth data:

- **Sitting height percentage** = (sitting height / height)*100
- **BMI** = weight(kg) / (height(m))^2
- **Lower Limb Length Ratio** = lower limb length / height
- **Leg Length =** height – sitting height
- **Relative Leg Length** = height-adjusted standardised residuals of leg length

Relative leg length was calculated by regressing leg length by sitting height to obtain the regression residual. This was then divided by the standard error of the estimate of the regression

equation to obtain height-adjusted standardised residuals. This method eliminates the need for z scores based on reference tables and the need to divide leg length by height when leg length has already been calculated based on height. Raw values of MUAC and head circumference were used in this analysis as growth curves show very little increments in either head circumference or MUAC from 2 to 11 years of age[214, 215].

4.4 Results

4.4.1 Missing Data

Besides deaths and loss to follow-up (already described in Chapter 3, section 3.1.1), the most significant missing data to mention for the growth outcomes is WAZ. WAZ in all children above 10 years of age is missing as the WHO 2007 growth reference, although range up to 19 years old for HAZ and BAZ, only extends to 10 years of age for WAZ. The WHO states that WAZ is not a good indicator in children above 10 years of age as it cannot distinguish between height and body mass in an age period where many children are experiencing the pubertal growth spurt and may appear as having excess weight using this measure, when in fact they are just tall[213]. There is no significant difference in amount of missing WAZ values between cases and community control, however there are significantly more missing WAZ values for sibling controls (p<0.001) as they are older on average than cases.

Erroneous data entry or measurements resulted in a very small amount of missing data for some anthropometry variables, as did children refusing to cooperate or children not attending the hospital appointments. For example, HAZ, had no significant difference in the prevalence of missing values between cases and siblings but there were significantly more missing values for community controls (p=0.006) due to a greater number of missed hospital appointments among community controls.

For a full table of number of results for each outcome variable see **Table 5** in Chapter 3: Section 3.1

4.4.2 Growth: Whole Cohort

The distributions of WAZ, HAZ and BAZ for the whole sample are shown in **Figure 12**. Although the sample is normally distributed, the majority of the children, including controls, have growth indicators below the global norm (z score 0) based on the WHO 2007 growth reference. BAZ is closest to global norm, followed by WAZ and then HAZ. **Table 10** shows proportion of each group with low (<-2) WAZ, HAZ and BAZ. There was no significant difference in z scores between the sexes within the study groups.

Histograms for each of the growth outcomes for the cohort as a whole can be found in **Appendix 13**.

% with low:	Cases	Sibling controls	Community controls
WAZ	33% (71/215)	23% (22/94)	21% (24/117)
HAZ	41% (123/299)	33% (68/203)	25% (39/159)
BAZ	9% (26/297)	11% (23/203)	8% (12/159)

Table 10: Proportion of children with low (<-2) WAZ, HAZ and BAZ in each study group

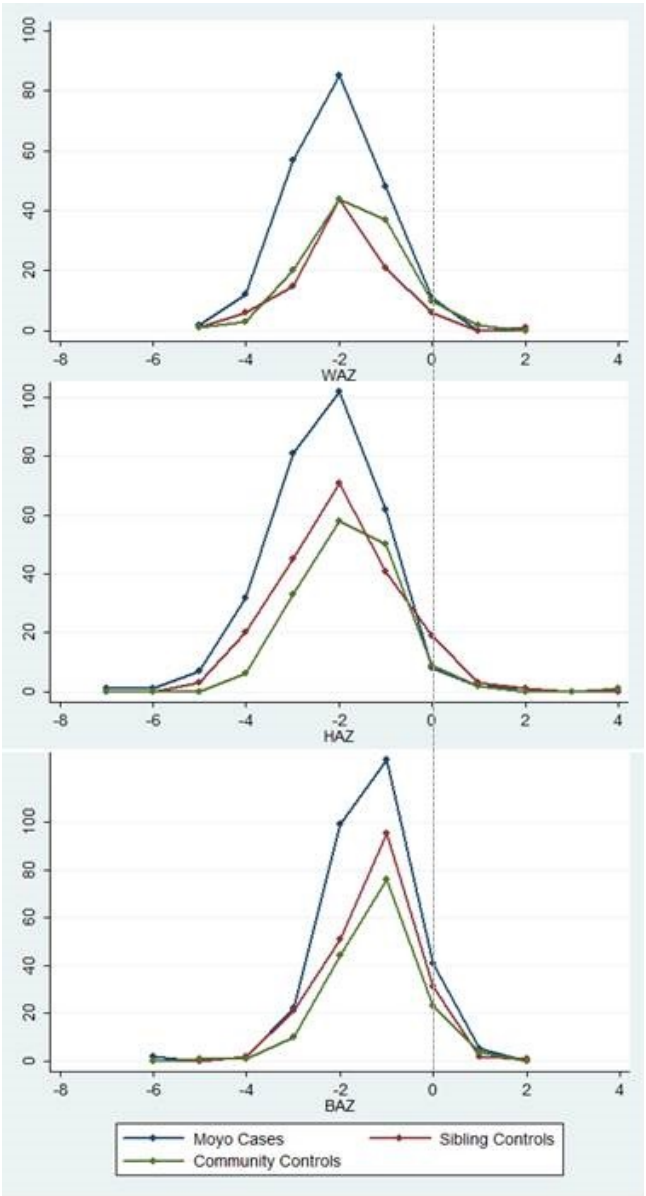


Figure 12: Frequency distribution for WAZ, HAZ and BAZ for the whole sample

4.4.3 Growth of Controls vs Cases

I hypothesised that cases would have impaired growth as compared to controls due to disrupted development during their episode of SAM and subsequent adaptations. Compared to controls, even in this group of healthy survivors, children who have had SAM have some compromised growth at 7 years post-discharge.

Table 11 shows means for each growth measurement and study group, and the results of both simple and multivariable linear regression analysis. In summary, the following growth outcomes are significantly smaller for cases than both sets of controls in adjusted analysis ($p<0.05$):

- weight
- standing height
- leg length
- relative leg length
- lower limb length ratio
- HAZ
- MUAC

Sitting height percentage is significantly larger for cases than both sets of controls. Interestingly, absolute sitting height is not significantly different between cases and controls, even though controls are significantly taller.

There is a marginal difference in WAZ between case children and community controls, but not sibling controls after adjusting for potential confounders. There is a significant difference in BMI between cases and sibling controls, but when converted to BAZ, there is no significant difference between either of the groups. **Figure 13** shows box plots for BAZ, HAZ and WAZ between the 3 groups. There is no significant difference between cases and either of the groups for head circumference.

Results are very similar when comparing HIV negative children only across the study groups, as can be seen in table in **Appendix 14**. There are a few differences worth mentioning; interestingly sitting height is significantly larger for both sibling and community controls compared to cases when looking at HIV negative children only but sitting height percentage is still significantly different between cases and community controls. Sibling controls also have significantly larger HAZ than cases when comparing HIV negatives only. These results indicate the negative effects that HIV is having on height, sitting height and sitting height ratio of the sibling controls. Case also have significantly smaller head circumference than community controls when looking at HIV negative children only. Head circumference is actually larger for HIV negative case group but confidence interval is smaller, affecting the p value. HIV may be masking some small differences in head circumference between cases and community controls due to larger range of measures.

Anthropometry	Cases	Sibling			Community		
	Mean (SD)	Mean (SD)	Unadjusted difference (95% CI) *P value*	Adjusted difference (95% CI) *P value*	Mean (SD)	Unadjusted difference (95% CI) *P value*	Adjusted difference (95% CI) *P value*
Weight							
Weight (kg)	23.9 (4.9)	27.7 (9.7)	3.8 * (2.5, 5.0) *<0.001*	1.9 * (1.0, 2.7) *<0.001*	25.4 (6.6)	1.4 * (2.5, 5.0) *0.04*	1.5 * (0.6, 2.4) *<0.001*
BMI	15.2 (1.4)	15.7 (1.9)	0.5 * (0.2, 0.8) *<0.001*	0.3 * (0.0, 0.6) *0.03*	15.4 (1.7)	0.2 (-0.2, 0.5) *0.31*	0.2 (-0.1, 0.5) *0.23*
Height							
Standing (cm)	124.9 (9.0)	130.3 (16.8)	5.3 * (3.1, 7.5) *<0.001*	2.0 * (0.6, 3.4) *0.006*	127.4 (9.9)	2.4 * (0.1, 4.8) *0.04*	2.7 * (1.2, 4.2) *0.001*
Sitting (cm)	65.2 (4.4)	67.4 (7.6)	2.2 * (1.2,3.2) *<0.001*	0.6 (-0.1,1.3) *0.11*	65.9 (4.5)	0.8 (-0.3,1.9) *0.18*	0.7 (-0.1,1.5) *0.07*
Sitting Height %	52.2 (1.5)	51.8 (1.7)	-0.4 * (-0.7, -0.1) *0.004*	-0.3 * (-0.6,-0.0) *0.02*	51.8 (1.5)	-0.5 * (-0.8,-0.1) *0.003*	-0.6 * (-0.9,-0.0) *<0.001*
Leg length (cm)	59.6 (5.5)	63.0 (9.6)	3.1 * (1.8, 4.4) *<0.001*	1.4 * (0.5, 2.3) *0.002*	61.6 (6.0)	1.7 * (1.8, 4.4) *0.02*	2.0 * (1.0, 3.0) *<0.001*
Relative leg length	39.9 (18)	49.6 (32.9)	9.7 * (5.4,14.0) *<0.001*	3.1 * (0.2,5.9) *0.04*	44.0 (19.2)	4.0 (-0.7,8.7) *0.09*	4.1 * (1.0,7.2) *0.01*
Lower limb length ratio	31.0 (1.6)	31.6 (1.8)	0.6 * (0.3, 0.9) *<0.001*	0.5 * (0.1, 0.8) *0.004*	31.5 (1.6)	0.5 * (0.1, 0.8) *0.005*	0.5 * (0.1, 0.8) *0.007*
Growth Z scores							
WAZ	-1.6 (0.9)	-1.4 (1.0)	0.1 (-0.1, 0.4) *0.27*	-0.2 (-0.5, 0.1) *0.16*	-1.2 (0.9)	0.4 * (0.0, 0.5) *0.002*	0.3 (-0.0, 0.5) *0.06*
BAZ	-0.8 (0.9)	-0.8 (0.9)	0.03 (-0.1, 0.2) *0.71*	0.08 (-0.1, 0.3) *0.39*	-0.7 (0.9)	0.1 (-0.0, 0.3) *0.14*	0.1 (-0.1, 0.3) *0.31*
HAZ	-1.8 (1.2)	-1.5 (1.2)	0.3 * (0.1, 0.5) *0.01*	0.2 * (0.0, 0.4) *0.04*	-1.3 (1.1)	0.5 * (0.3, 0.7) *<0.001*	0.4 * (0.2, 0.6) *<0.001*
Body circumferences							
Head (cm)	51.1 (2.1)	52.1 (2.5)	0.4 * (0.0, 0.7) *0.04*	0.1 (-0.3, 0.5) *0.37*	52.1 (1.9)	0.3 (-0.1, 0.7) *0.21*	0.3 (-0.1, 0.8) *0.12*
MUAC (mm)	172 (20)	183 (29.8)	10.9 * (6.7, 15.1) *<0.001*	5.7 * (2.3, 9.1) *0.001*	178 (22)	5.5 * (1.0, 10.1) *0.02*	5.6 * (1.9, 9.4) *0.003*

Table 11: Results of simple and multivariable linear regression analysis, comparing siblings and community controls to cases for each growth variable at 7 years post-discharge. Adjusted difference includes age, sex, HIV status, SES and puberty in regression model. * indicates *p* value less than 0.05. Sitting height % = sitting height/height*100; Relative leg length = regression residuals for leg length by sitting height; Lower limb length ratio = lower limb length/height

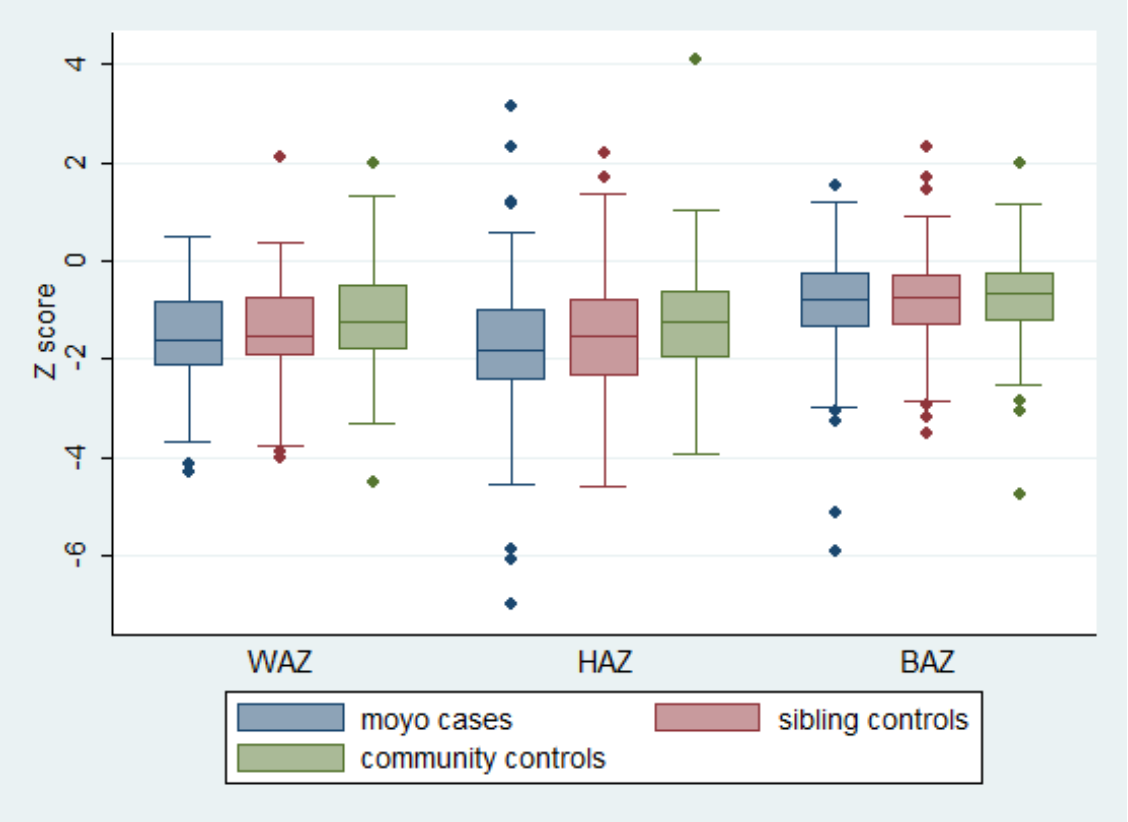


Figure 13: Box plots showing mean weight-for-age, height-for-age and BMI-for-age z scores for each of the 3 study groups: cases, siblings and community controls at 7 years post-discharge.

4.4.4 Longitudinal Growth Data

Using growth data across four time points from admission to 7 years post-discharge for case children, results suggest that case children are catching up towards global norms for weight and height. The FuSAM study found very little improvement in HAZ from discharge to 1 year post-discharge, however I have found a large relative improvement in HAZ from 1 year post discharge to 7 years post discharge, as seen in **Table 12**. Conversely, WAZ made large gains in the first year post-discharge but has since slowed. BMI-for-age deteriorated from 1 year post-discharge to present, due to the large gains in height not being matched by gains in weight. The differences in BAZ WAZ and HAZ across 3 time points are depicted as boxplots in **Figure 14**. Some studies have assessed catch up growth by comparing the change in proportion of stunting in the group over time[103]; in this study, at 1 year post-discharge 83% (249/299) children were stunted (HAZ <-2) while at 7 years post-discharge 41% (123/299) of the case children are stunted. We can compare these proportions to sibling controls where 46% (140/302) were stunted in 2006, and now 33% (68/203) of siblings are stunted.

Using "spaghetti" plots focusing on those children who were in the smallest or largest 10% during admission, we can see that those starting from lower z scores have greater catch-up growth than those starting with less of a deficit. **Figure 15** shows 3 spaghetti plots for WAZ, HAZ and BAZ across 5 time points: admission, minimum weight on the ward, discharge from OTP programme, 1 year post-discharge and 7 years post-discharge. Although those with low z score have shown the most improvements, there are very few children who have crossed-over from smallest to largest and vice-versa. This suggests that those children who were small during admission have exhibited catch up growth but still remain relatively small at 7 years post-discharge

	Mean (SD) on ward	Mean (SD) discharge	Mean (SD) 1 year p.d.	Mean (SD) 7 years p.d.	Difference discharge - 1year	Difference 1year – 7 years
WAZ	-3.76 (1.4)	-2.63 (1.5)	-1.75 (1.2)	-1.56 (1.0)	0.88	0.19
HAZ	-3.24 (1.4)	-3.31 (1.7)	-3.28 (1.3)	-1.82 (1.2)	0.03	1.46
BAZ	-2.67 (1.7)	-0.73 (1.4)	0.65 (1.4)	-0.85 (0.9)	1.38	-1.50

Table 12: Mean (SD) of WAZ HAZ and BAZ across 4 time points (on the ward, at discharge from OTP, at 1 year post-discharge and at 7 years post-discharge) (p.d = post-discharge). Difference in z score between discharge and 1 year post-discharge, and difference in z score between 1 year post-discharge and 7 years post-discharge are also presented.

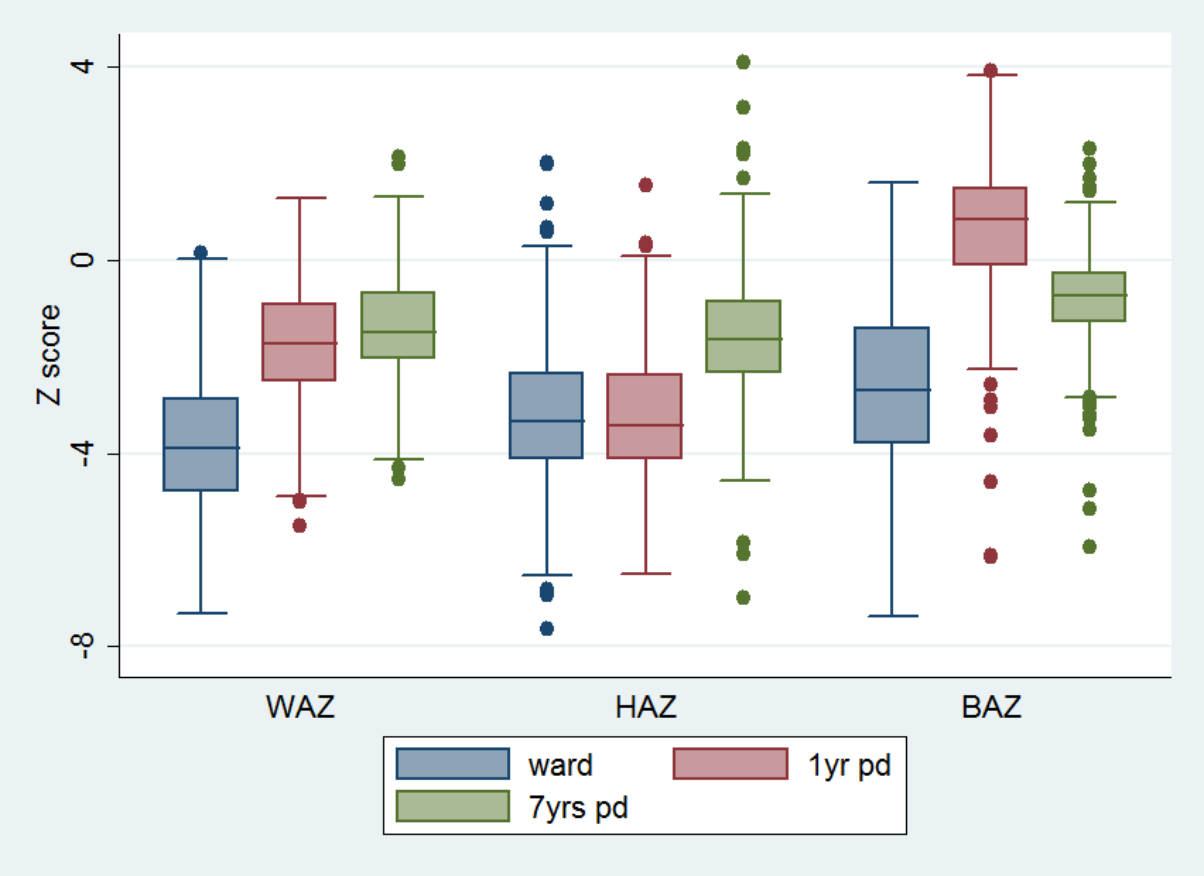
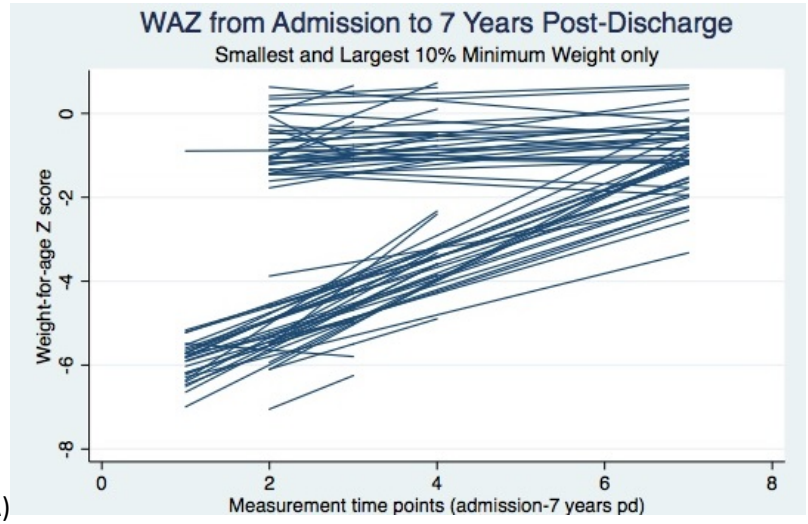
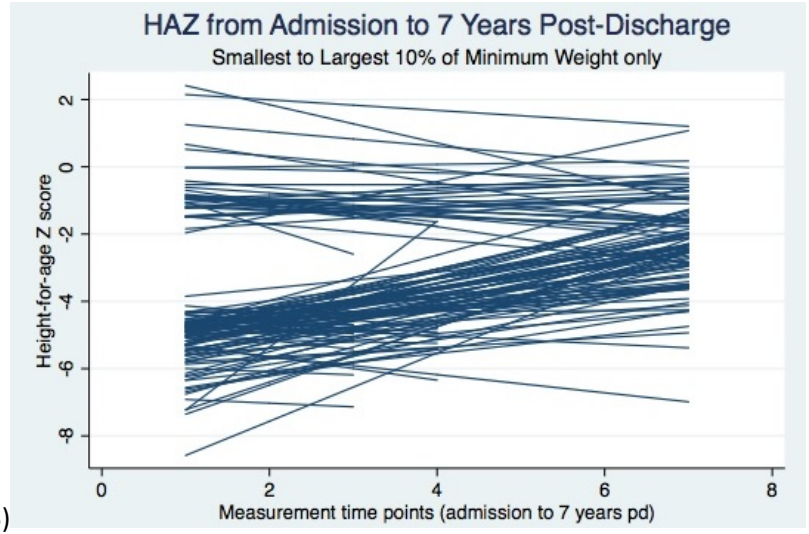


Figure 14: The differences in WAZ, HAZ and BAZ for cases across the 3 time points: at their minimum weight on the ward, at 1 year post-discharge and at 7 years post-discharge.

A)



B)



C)

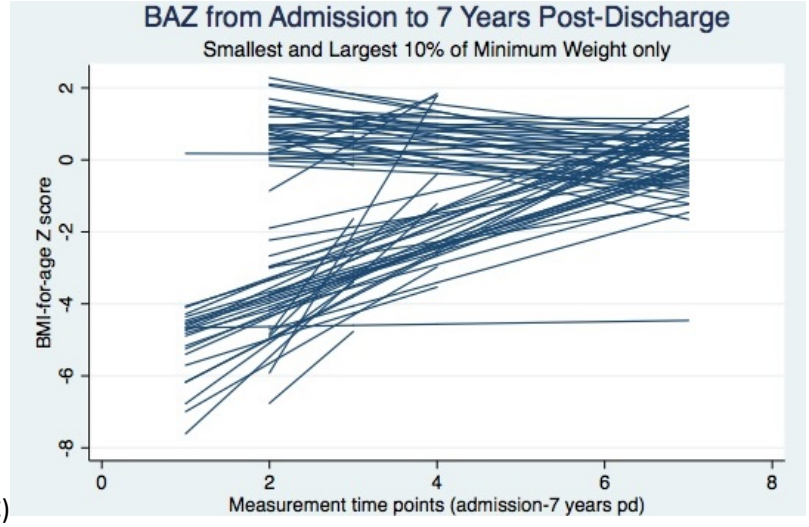


Figure 15: Spaghetti plots showing changes in a) WAZ, b) HAZ and c) BAZ for individual children from admission, at minimum weight during admission, discharge from OTP programme, 1-year post-discharge and 7-years post discharge. This is for the smallest 10% and largest 10% at minimum weight on the ward only.

4.4.5 Longitudinal Data: Comparing Growth of Cases with Sibling Controls

As some sibling controls were recruited at 1 year post-discharge, I could compare the changes in WAZ, HAZ and BAZ of cases to siblings between 1 year and 7 years post-discharge. Results of "repeated measures ANOVA" show that there is both a significant difference in HAZ and BAZ within each study group between 1 year and 7 years post-discharge (p<0.001 for both), and more importantly, sibling controls have significantly larger HAZ, BAZ and WAZ than cases across the time periods (significant difference between the groups) (p <0.001, 0.02 and <0.001 respectively). Repeated measures mixed regression models can also be used in place of ANOVA, giving the same results; it confirms that the adjusted change in HAZ across the time points was 1.1 z-scores greater in cases than sibling controls, and change in WAZ was 0.9 z-scores greater (p<0·001 and <0·01 respectively). **Figure 16** shows modelled means for HAZ and WAZ allowing us to see how means of sibling and case groups have changed between 1 year and 7 year post-discharge follow ups. Interestingly mean WAZ for siblings dropped slightly whereas cases have increased. Both groups have increased their HAZ though change in HAZ is steeper for cases than controls. It is important to note that although CIs on the graphs do overlap, this does not mean there is no significant difference between the groups; the CIs are for point estimates, not differences. This data suggests that cases are catching up their lost WAZ and HAZ at a faster rate than sibling controls.

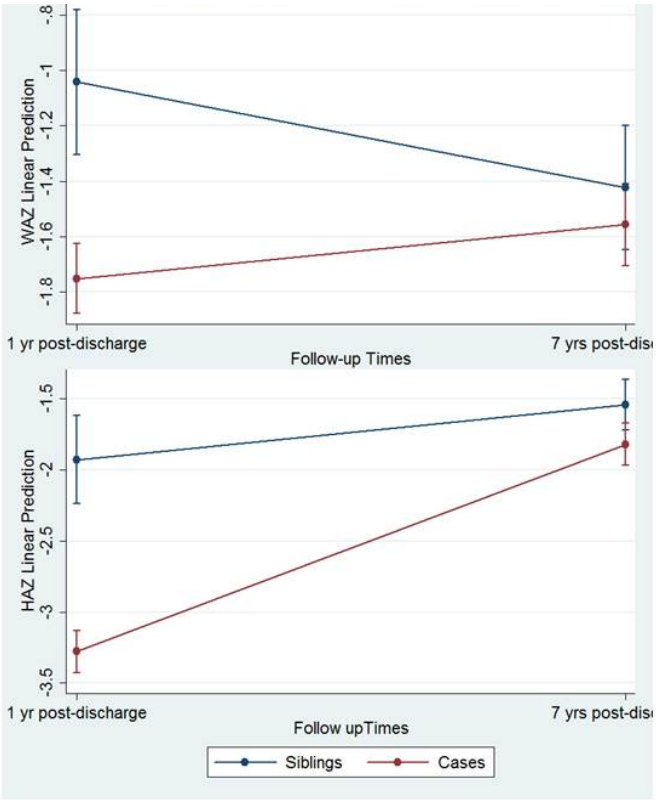


Figure 16: Modelled means for HAZ and WAZ for siblings and cases at 1 and 7 years post-discharge, including 95% confidence intervals.

4.4.6 Impact of HIV on Growth

HIV has had a significant impact on growth in SAM survivors, particularly affecting the prevalence of stunting. Mean values and SD scores for anthropometric growth measures are presented in **Table 13**, stratified by study group and HIV status. Notice that the number of HIV positive children in the two control groups is small. The difference between HIV positive and HIV negative groups is most notable for HAZ (unadjusted difference -0.60, 95% CI -0.9 to -0.3, p<0.001). WAZ, BAZ, sitting height ratio, head circumference and MUAC show no significant difference between HIV positive and negative groups. **Figure 17** shows mean HAZ for each study group stratified by HIV status; notice that HIV positive sibling controls actually have the lowest HAZ, which could be evidence of survivor bias.

	Cases		Sibling Controls		Community Controls	
	HIV positive	HIV negative	HIV positive	HIV negative	HIV positive	HIV negative
n	90	208	9	130	5	95
WAZ	-1.7 (1.0)	-1.5 (0.9)	-1.8 (0.3)	-1.5 (1.1)	-1.0 (1.9)	-1.2 (1.1)
HAZ	-2.1 (1.2)	-1.7 (1.2)	-2.3 (0.7)	-1.4 (1.2)	-1.6 (0.6)	-1.2 (1.2)
BAZ	-0.9 (0.9)	-0.8 (1.0)	-0.7 (0.5)	-0.8 (0.9)	-0.8 (1.4)	-0.7 (1.0)
Sitting height %	52.3 (1.4)	52.2 (1.5)	52.4 (1.4)	51.7 (1.7)	49.8 (3.1)	51.9 (1.3)
Lower limb ratio	31.0 (1.2)	31.0 (1.8)	30.0 (3.2)	31.7 (1.7)	32.9 (2.1)	31.6 (1.1)
Head circumference (cm)	52.0 (1.8)	51.7 (2.2)	51.1 (1.2)	51.9 (2.6)	52.0 (1.2)	52.0 (1.9)
MUAC (mm)	174.3 (21)	171.5 (19)	168.9 (20)	183.6 (33)	171.8 (5.9)	176.5 (23)

Table 13: Mean values and SD scores for growth variables by study group and HIV status. Those with unknown HIV status are not included. Sitting height % = sitting height/height*100. Lower limb ratio = lower limb length/height.

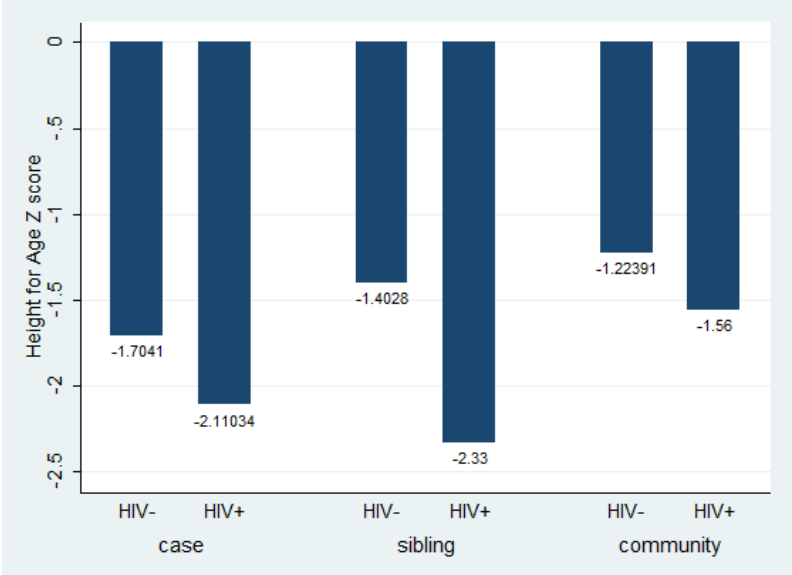


Figure 17: Bar graph showing mean HAZ by study group and HIV status at 7 years post-discharge

4.4.7 Predictors of Growth Outcomes

Lastly, I explored factors at admission which might predict poorer growth outcomes at 7 years post-discharge. **Table 14** presents raw data (means (SD)) for growth outcomes of children with different characteristics at admission. Further analysis show that small WAZ, HAZ and BAZ at admission, no oedema and being older than 2 years at admission are associated with worse growth outcomes at 7 years post-discharge. Time taken to recover is not associated with 7-year growth outcomes. Reported birth size of cases is also not associated with any long-term growth outcomes except head circumference; results of these analyses are presented in more detail below.

Mean (SD)	WAZ	HAZ	BAZ	Sitting height %	Head circumferenc
Degree of wasting at admission					
Low WAZ <-4 (n=137)	-1.85 (1.0)	-2.17 (1.3)	-1.06 (1.0)	52.3 (1.5)	51.6 (2.0)
High WAZ >-4 (n=160)	-1.29 (0.9)	-1.52 (1.0)	-0.67 (0.8)	52.2 (1.5)	52.1 (1.7)
Degree of stunting at admission					
Low HAZ (<-3) (n=164)	-1.87 (0.9)	-2.28 (1.1)	-0.93 (0.9)	52.2 (1.6)	51.7 (1.9)
High HAZ (>-3) (n=132)	-1.21 (0.9)	-1.23 (1.0)	-0.72 (0.9)	52.3 (1.4)	52.1 (1.7)
Kwashiorkor or wasting					
Oedema (n=265)	-1.49 (0.9)	-1.79 (1.2)	-0.79 (0.9)	52.2 (1.5)	51.9 (1.8)
No oedema (n=54)	-1.82 (1.0)	-2.00 (1.3)	-1.10 (1.0)	52.3 (1.4)	51.8 (2.0)
Time to recovery					
<50 days (n=)	-1.44 (0.9)	-1.75 (1.2)	-0.81 (1.0)	52.2 (1.5)	51.9 (1.8)
>50 days (n=)	-1.76 (1.0)	-1.95 (1.2)	-0.92 (0.8)	52.4 (1.4)	51.7 (1.9)
Age at admission					
<2 years (n=157)	-1.53 (1.0)	-1.51 (1.1)	-0.79 (0.9)	52.4 (1.4)	51.6 (1.9)
>2 years (n=142)	-1.63 (0.9)	-2.17 (1.2)	-0.92 (0.9)	52.0 (1.5)	52.1 (1.8)
Reported birth size					
Small at birth (n=33)	-1.70 (1.0)	-1.73 (0.9)	-1.10 (1.0)	52.1 (1.6)	50.8 (2.2)
Normal size at birth (n=332)	-1.54 (1.0)	-1.84 (1.2)	-0.82 (0.9)	52.2 (1.5)	52.0 (1.8)

Table 14: Means and standard deviations for main growth outcomes stratified by characteristics at admission

4.4.7.1 Degrees of Stunting and Wasting at admission

Linear regression analysis confirms that degree of nutritional deficiency at admission is predictive of some growth measures at 7 years post-discharge, as seen in **Table 15**. The only exceptions are that BAZ during admission does not predict degree of stunting (HAZ) or head circumference 7 years post-discharge, and neither WAZ, HAZ nor BAZ during admission predict sitting height percentage at 7 years post-discharge.

	WAZ during admission		**HAZ during admission**		**BAZ during admission**	
	Adjusted difference (95% CI)	P value	Adjusted difference (95% CI)	P value	Adjusted difference (95% CI)	P value
WAZ 7yrs post-discharge	0.29 (0.2, 0.4)	<0.001*	0.32 (0.2, 0.4)	<0.001*	0.11 (0.0, 0.2)	0.009*
HAZ 7yrs post-discharge	0.28 (0.2, 0.4)	<0.001*	0.42 (0.4, 0.5)	<0.001*	-0.01 (-0.1, 0.1)	0.73
BAZ 7yrs post-discharge	0.21 (0.1, 0.3)	<0.001*	0.11 (0.0, 0.2)	0.006*	0.15 (0.1, 0.2)	<0.001*
Sitting height %	-0.05 (-0.2, 0.1)	0.45	-0.12 (-0.2, 0.0)	0.05	0.03 (-0.1, 0.1)	0.59
Head circumference (cm)	0.34 (0.2, 0.5)	<0.001*	0.34 (0.2, 0.5)	<0.001*	0.13 (-0.0, 0.3)	0.07

Table 15: Results of linear regression analysis assessing associations between nutritional z scores at the point of minimum weight during admission and growth outcomes at 7 years post-discharge. Outcome at 7 years post-discharge is the dependant variable. Regression has been adjusted for HIV status, age, sex and SES. * indicates $p<0.05$. Sitting height % = sitting height/ height *100.

4.4.7.2 Oedema

Presence of oedema at admission is associated with better growth outcomes at 7 years post discharge. Those who didn't have oedema at admission had a worse WAZ, HAZ and BAZ at admission, and continued to have a worse HAZ at 1 year post-discharge, as previously discussed by Kerac et al[87]. Comparison of mean z scores of cases with and without oedema, as well as comparison of these two groups with controls, shows a small shift compared to the previous follow up. Those without oedema no longer have significantly lower HAZ at 7 years post-discharge, but they do have significantly lower WAZ and BAZ. **Table 16** shows results of multivariable linear regression analysis comparing those admitted with oedema and those admitted without to the control group (sibling and community combined). Control groups are combined in this instance as larger numbers increase power of the analysis. Results show that both oedematous and non-oedematous admissions have significantly larger sitting height percentage than controls, but they are not significantly different from one another. There is no significant difference between either group and controls for head circumference. It is important to remember that oedema is much more prevalent than wasting in this cohort. **Figure 18** shows spaghetti plots for WAZ and HAZ comparing the oedematous and non-oedematous groups over the course of follow up; this highlights that although WAZ in the non-oedema group started significantly lower and remains significantly lower, the has been significant catch-up growth in both groups. This is also true for HAZ, although the differences are less pronounced.

	Survivors with Oedema (n= 265)		Survivors without Oedema (n= 54)	
	Regress coef. (95% CI)	P value	Regress coef. (95% CI)	P value
WAZ	-0.11 (-0.32, 0.09)	0.28	-0.45 (-0.80, -0.09)	0.01*
HAZ	-0.27 (-0.47, -0.07)	0.01*	-0.43 (-0.80, -0.06)	0.02*
BAZ	-0.01 (-0.17, 0.15)	0.90	-0.40 (-0.69, -0.10)	0.01*
Sitting height %	0.49 (0.21, 0.77)	<0.001*	0.51 (-0.00, 1.02)	0.05*
Head circumference	0.04 (-0.63, 0.70)	0.91	0.37 (-0.18, 0.93)	0.18

Table 16: Results of linear regression analysis for growth outcomes at 7 years post-discharge comparing cases who had oedema at admission and those who didn't, with controls (siblings and community combined), adjusted for HIV and SES. * indicates significant difference. Sitting height % = sitting height/height*100.

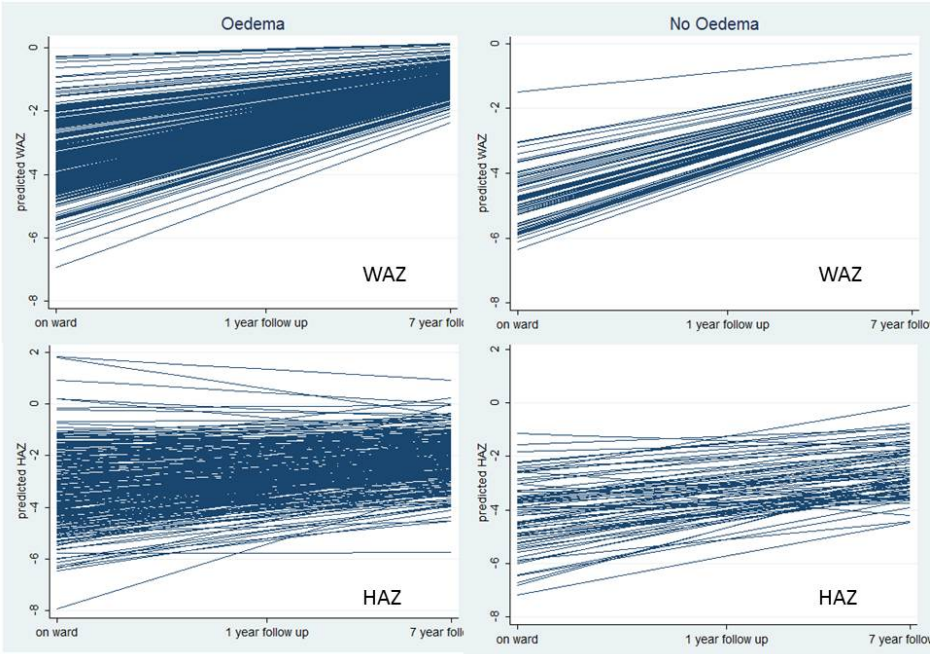


Figure 18: Spaghetti plots for WAZ and HAZ for those who did and didn't have oedema at admission, over the course of follow up.

4.4.7.3 Time to recovery

I also assessed whether time taken to recover was associated with long-term growth; I found no significant association between time taken to recover and BAZ, WAZ or HAZ at 7 years post-discharge. **Figure 19** shows scatter graphs of WAZ and HAZ at 7 years post-discharge plotted against days taken to recover for SAM.

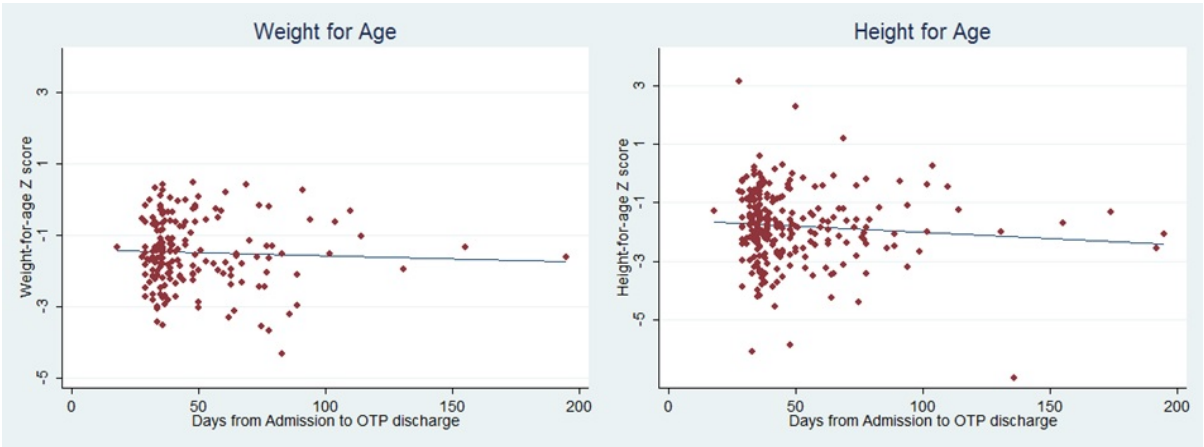


Figure 19: Scatter plots of WAZ and HAZ at 7 years post-discharge against number of days taken to recover (admission to discharge from OTP). The regression line is not significant for either graph.

4.4.7.4 Age at Admission

I also assessed whether age at admission had an impact on growth outcomes, and found that HAZ, MUAC and BAZ are significantly smaller in children admitted at an older age. WAZ, sitting height percentage and head circumference are not affected by the age at admission. Physical disability and HIV are associated with older age at admission therefore they are adjusted for in the regression model. Low HAZ is also associated with increased age at admission however adjusting for HAZ at admission does not remove the significant correlation between age at admission and HAZ at 7 years post-discharge. I can therefore conclude that children admitted when they are older are more likely to be more stunted on admission and for every month increase in age at admission they are likely to be 0.02 z scores more stunted at follow up ($p<0.001$), regardless of how stunted they were at admission.

Table 17 shows results of multivariable linear regression analysis comparing growth outcomes of those who were admitted at or before 24 months and those who were admitted after 24 months to controls. This analysis confirms that older admissions have worse HAZ outcomes as those admitted after 24 months have significantly lower HAZ than controls, whereas those admitted before 24 months do not have a significantly different HAZ to controls. Young admissions seem therefore to have caught up to community HAZ levels. Both those admitted before and those admitted after 24 months have significantly larger sitting height percentage than controls.

I considered whether HAZ and age at admission had an n-shaped relationship i.e. the very young ones have worse outcomes, and as do the very old ones. However the line graph in **Figure 20** shows that this is not the case; with every categorical increase in age at admission, the mean HAZ at follow-up gets worse. There could be an element of survivor bias raising the HAZ of the youngest admission category (0-12months), as this group had a significantly heightened risk of mortality, and only 33% of this group were alive at 2-years post-admission.

	Survivors admitted before or at 24 months (n=188)		Survivors admitted after 24 months (n=172)	
	Regress coef. (95% CI)	P value	Regress coef. (95% CI)	P value
WAZ	-0.12 (-0.33, 0.1)	0.28	-0.23 (-0.52, 0.06)	0.12
HAZ	-0.03 (-0.26, 0.2)	0.81	-0.62 (-0.86, -0.37)	<0.001*
BAZ	-0.03 (-0.21, 0.16)	0.76	-0.14 (-0.34, 0.06)	0.18
Sitting height %	0.31 (0.01, 0.61)	0.04*	0.58 (0.026, 0.90)	<0.001*
Head circumference	-0.04 (-0.45, 0.38)	0.86	-0.44 (-0.88, 0.01)	0.05
MUAC	-5.15 (-8.79, -1.52)	0.01*	-7.11 (-11.1, -3.21)	<0.001*

Table 17: Results of linear regression analysis comparing growth outcomes of those who were admitted before or at 24 months and those who were admitted after 24 months to controls, adjusted for HIV status, age, sex, SES and physical disability.

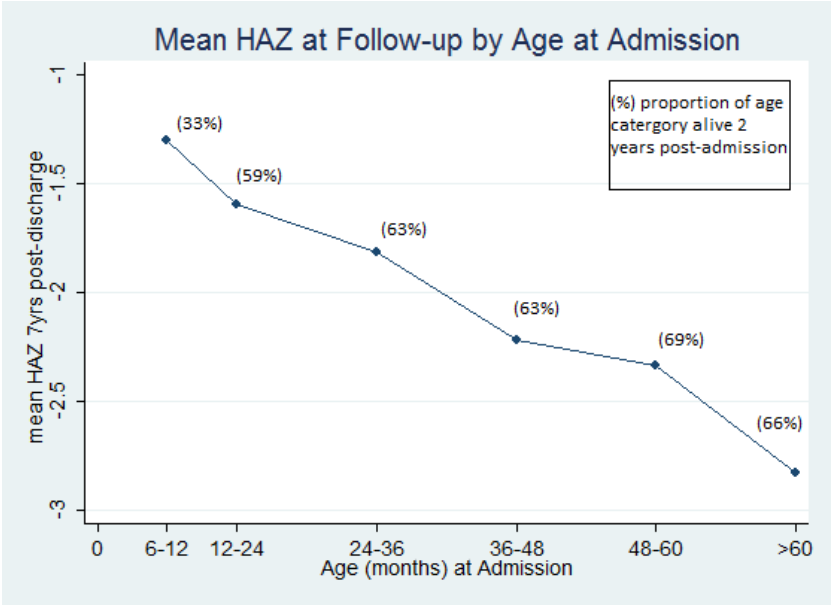


Figure 20: Line graph showing mean HAZ at ChroSAM 7 year follow-up for each "age at admission" category. Percentages next to each point indicate the proportion of that age category that survived SAM treatment as a consideration of survivor bias.

4.4.7.5 Reported Birth Weight

Birth weight is often used as an indicator for prenatal nutrition; prenatal nutrition is known to have long-term consequences on growth and other health outcomes. Birth weight was not routinely recorded in medical documents when the majority of this cohort were born however during PRONUT study mothers were asked to give an estimate of the size of their child at birth: normal, small or very small.

I assessed whether estimated size at birth was associated with main growth outcomes at 7 years post-discharge. Numbers in the "small at birth" category were small, however I found no significant difference between though who were normal size at birth and those who were small or very small at birth for WAZ, HAZ, BAZ and sitting height percentage at 7 years post-discharge. There was an association between being small at birth and small head circumference in childhood (adjusted difference -0.93, 95% CI -1.7 to -0.2, p=0.01). When the two types of birth size are compared to controls (**Table 18**), only those small at birth had significantly smaller BAZ and head circumference. Interestingly only those who were normal-large size at birth had significantly smaller HAZ and larger sitting height percentage than controls.

	Normal size at birth (n= 288)		**Small size at birth (n= 72)**	
	Regress coef. (95% CI)	P value	Regress coef. (95% CI)	P value
WAZ	-0.06 (-0.3, 0.2)	0.57	-0.27 (-0.7, 0.2)	0.26
HAZ	-0.30 (-0.5, -0.1)	0.00 *	-0.25 (-0.7, 0.2)	0.30
BAZ	-0.05 (-0.2, 0.1)	0.53	-0.43 (-0.8, -0.0)	0.03 *
Sitting height %	0.46 (0.2, 0.7)	0.00 *	0.12 (-0.5, 0.7)	0.69
Head circumference	-0.17 (-0.5, 0.1)	0.30	-1.07 (-1.8, -0.3)	0.01 *

Table 18: Results of linear regression analysis for growth outcomes at 7 years post-discharge comparing cases that were reportedly small or very small at birth with those who were normal or large size at birth, with controls (siblings and community combined), adjusted for HIV, age, sex and SES. * indicates p<0.05. Sitting height % = sitting height/height*100.

4.4.8 Summary

This chapter explored various aspects of growth including weight-for-age, height-for-age, BMI-for-age, sitting height proportions, leg length and head circumference. Results show that majority of the children in the sample are stunted and underweight compared to global norms, however SAM survivors are significantly more stunted than both their siblings and children of similar age in their community. SAM survivors also have similar sitting height and significantly shorter legs than controls, resulting in a significantly larger sitting height percentage. Longitudinal analysis of the change in HAZ since 1 year post-discharge suggests that case children are catching up their linear growth at a steeper rate than their sibling controls. These results also highlight the significant adverse impact that HIV has had on linear growth independently of SAM; however the long-term effects of SAM on linear growth are still significantly evident once HIV has been either adjusted for or removed from the analysis. Other factors at admission have impacted long-term growth; namely small WAZ, HAZ and BAZ at admission, no oedema and being older than 2 years at admission are associated with worse growth outcomes at 7 years post-discharge. Reported birth size of cases is associated with head circumference at 7-years post-discharge, but not other growth outcomes.

5 Chapter 5: Body Composition

This chapter explores the potential effects that SAM could have on long-term body composition. Body composition as well as growth is an important component of health; and where growth tells us about absolute size, body composition allows for differentiation between fat and lean mass and its distribution across the body. Measures of body composition used in this chapter are waist, hip and calf circumference, bioelectrical impedance analysis (BIA) and skinfold thickness. Following an introduction to previous studies and existing knowledge, body composition methods are described and results of analysis presented. Primary analysis compares body composition outcomes of cases to controls to assess whether an episode of SAM has had enduring detrimental effects in this area; secondary analysis explored predictors of poor body composition in case children.

5.1 Chapter-specific Hypotheses

As described in the Chapter 1, body composition is a measure of the amount and distribution of fat and fat-free mass in the body, as well as the body's general size and shape. Not only amount of fat, but positioning of fat is of great importance to health, particularly NCDs in adulthood [130-132]. Barker, in his proposal of the "Thrifty Phenotype" hypothesis, suggested that the adaptive phenotype during undernutrition favours fat storage, particularly on the core body where resources are needed for vital organ function and development, and this phenotype continues post-malnutrition[29]. Based on this theory and evidence from low birth weight studies, I hypothesised the following:

3. Compared to controls, SAM survivors have an altered body composition, specifically (a) greater proportion of central fat to peripheral fat, (b) a lower magnitude of lean mass.

5.2 Measuring Body Composition

There are various ways of measuring body composition, beside the gold standard cadaver analysis. Each in vivo technique however makes assumptions as they do not measure body composition directly but predict it based on different body properties. Techniques for measuring body composition include underwater weighing (densitometry), dual energy x-ray absorptiometry (DEXA), anthropometry, bioelectrical impedance analysis (BIA), magnetic resonance imaging (MRI) and skinfold thickness. The relative benefits of different techniques have been reviewed in detail[216]. No single technique is likely to be optimal in all circumstances therefore this study uses a number of different techniques, including anthropometry, skinfold thickness and bioelectrical impedance (BIA), selected based on both the reliability of the method and the practicalities of what was plausible in the study setting, with the study budget.

Anthropometry measures include calf, hip and waist circumference; waist circumference is indicative of core fat whereas hip circumference indicates peripheral fat. Waist/hip ratio is a commonly used measure of the amount of core fat in relation to peripheral fat. There is some debate as to whether waist/hip ratio or waist circumference is the better predictor of NCDs [217, 218]. It has also been noted that waist/hip ratio is not always a good measure of central fat if the waist girth alone is low; young children can have a small waist girth and still have a high waist/hip ratio, yet they do not have high central adiposity[219]. High waist/hip ratio can also be as a result of small hip circumference rather than large waist circumference, which is also relevant as small hip circumference is associated with higher risk of diabetes, independent of BMI and waist circumference[132]. In the absence of age and sex reference data for waist and hip circumference, one way of standardising waist and hip circumference measures is to divide by height; waist to height ratio is gaining evidence as a good measure of central adiposity[220]. Hence it is important to explore all these variables independently as well as in ratio form.

Skinfold thickness is also a commonly used measure of body fat distribution. It measures the amount of subcutaneous fat using callipers, and can be done at various standardized places on the body. Again, it is used to distinguish between core and peripheral fat distribution. Skinfold thickness of triceps and biceps indicate amount of peripheral fat, and measures of the waist and subscapular indicate level of central adiposity. Studies have also correlated skinfold thickness measures with impaired functional outputs and NCD risk; for example high subscapular skinfold readings are associated with persistent impaired glucose tolerance[221]. Skinfold thickness can be an inaccurate and imprecise measure in obese people however in others it is generally thought that intra- and inter-observer errors are low compared to between-subject variability. Skinfold thickness can be converted into percentage body fat but this is now thought to be relatively unreliable; it is now thought that raw values and z-scores based on reference data are the most

reliable use of skinfold thickness for determining regional fatness[216]. There is no reference data for skinfold thickness in sub-Saharan Africa, but it is possible to create reference data from the control children in the study using the LMS method in order to generate skinfold thickness z scores[222]. A ratio of core to peripheral skinfold measures can also be used as an indicator of core fatness, without the need for reference data, although some adjustment for age is recommended[223].

Lastly, BIA can be used to better understand weight as it estimates the proportions of water, fat and fat free mass which make up total body weight. BIA is based on the assumption that the body is a cylindrical shaped ionic conductor in which the extracellular and intracellular non-adipose tissue compartments act as resistors and capacitors, respectively[224]. BIA gives a measure of electrical impedance (Z) and its two components: resistance (R) and reactance (Xc). Resistance is the opposition offered by the body to the flow of an alternating electrical current, and it is inversely related to the water and electrolyte content of tissue. Reactance is related to the capacitance properties of the cell membrane, and variations can occur depending on its integrity, function, and composition[225]. Many researchers have developed empirical equations for predicting total body water (TBW), fat free mass (FFM), body cell mass and body fat content using weight, age, sex, race and other variables in addition to resistance and height[226-228]. TBW can be calculated using resistance(R), weight, height, sex and age. Once TBW is calculated, FFM can be calculated by diving by the "hydration constant". The equations are based on the fact that muscle has higher water content than fat mass; therefore higher TBW equates to higher percentage of your weight being composed of FFM. In the absence of a population-specific equation and "hydration constant", it is possible to calculate a proxy for lean mass by dividing impedance by height (impedance index). The residuals of BMI regressed on impedance index can provide a value proportional to fat mass index. These values do not have units in kilograms like actual FFM and FM but are good proximate values[229]. In relation to malnutrition, it is theorised that the "thrifty phenotype" will result in children exposed to nutritional insults having less FFM than controls due to inhibition of organ growth and muscle mass accumulation, and may also have higher FM due to hoarding of central fat. Because BIA does not distinguish between core fat and peripheral fat, it is not sure whether total fat mass will be higher in cases or controls in this study.

A common method for analysing BIA data in the absence of a population-specific empirical equation is to use isotope dilution methods to validate FFM calculations[216]. Isotopes of hydrogen (such as deuterium) can be used to quantify TBW and therefore this method can be used to calibrate the calculation of FFM from BIA[230]. However, the isotope mass spectrometry required to estimate TBW from isotope dilution is costly and time-intensive, and was not budgeted for as part of this study. Additionally, Piccoli (1994 & 2006) believes that isotope

dilution is not accurate enough in clinical settings where patients might have altered hydration status. He suggests BIA Vector Analysis (BIVA) as the best way to compare levels of lean mass and hydration using BIA data[231, 232]. As impedance is a direct measure of lean and fat mass combined, impedance vectors can be used on any population as a stand-alone procedure. This method plots R/height against Xc/height, and the arc tangent of Xc/R is called the phase angle. Long vectors with a small phase angle (increased R with decreased Xc) are associated with malnutrition, whereas short vectors with a small phase angle (decreased R with decreased Xc) are associated with fluid overload[232]. I will use this method to compare impedance readings between cases and controls.

The measure of 'Phase Angle', derived from the relation between the direct measures of resistance and reactance can also be analysed as a stand-alone outcome. Its biological meaning and pathogenic effects are not completely understood, however phase angle has been interpreted as an indicator of body cell mass, cell membrane integrity and water distribution within and between cells[233]. It may also be an indicator of function and general health, however this is still being explored[234]. There is currently evidence linking low phase angle with malnutrition, lower muscle strength and mortality risk in hospitalized patients[235, 236]. It might be that SAM survivors have a lower phase angle than control children as their cell integrity, general function and health has been disrupted by an insult in early growth and development.

5.3 Specific Methods

5.3.1 Anthropometry

Waist circumference: The child removed or lifted up any vest/shirt to expose their waist. The child stood straight with abdomen relaxed, arms hanging at their side and feet together. The operator located the waist by finding the top of the iliac crest. The *Rollfix* Hoechstmass measuring tape was passed around the waist and the reading taken at the end of a normal expiration.

Hip circumference: The child was measured standing and wearing thin clothing or underwear. Whilst squatting at the child's side, the operator found the widest girth of the hips, usually around the greatest trochanters of the femur, and passed the measuring tape around this point.

Calf circumference: The child stood stand straight with their weight evenly distributed over both legs. The calf circumference was measured on the right leg, at the widest point of the calf, with the measuring tape perpendicular to the long axis of the calf.

Waist circumference, hip circumference and calf circumference were all measured to the nearest 0.1cm.

Image 7: Measuring a child's calf circumference on the right leg

5.3.2 Bioelectrical Impedance (BIA)

BIA was measured on the children using the Quadscan 4000 machine (Bodystat Ltd, UK), as seen in **Image 8**. The Quadscan provides data on impedance, resistance, reactance and phase angle. This standard BIA technique, with tetrapolar measurement on a hand and a foot with 50 kHz current frequency, provides the best information at a body level because it maximizes the signal-to-noise ratio and minimizes variability of electric flow path of multi-frequency BIA[231].

The BIA readings were taken from the right side of the body while the child was lying on their back. Follow the manufacturer's instructions, the child's hands and feet were washed and dried, and their arms were at their side but not touching their sides; their thighs were also not touching one another. The electrodes were connected at the wrist, hand, ankle and foot in the positions seen in **Image 9**. A second reading was taken immediately after the first by removing and reattaching the electrode leads, but leaving electrode stickers in place. If the two readings varied by more than 20 ohms, the BIA data were deemed invalid and excluded from analysis.

Image 8: Bodystat Quadscan 4000 being used to measure BIA in Blantyre, Malawi

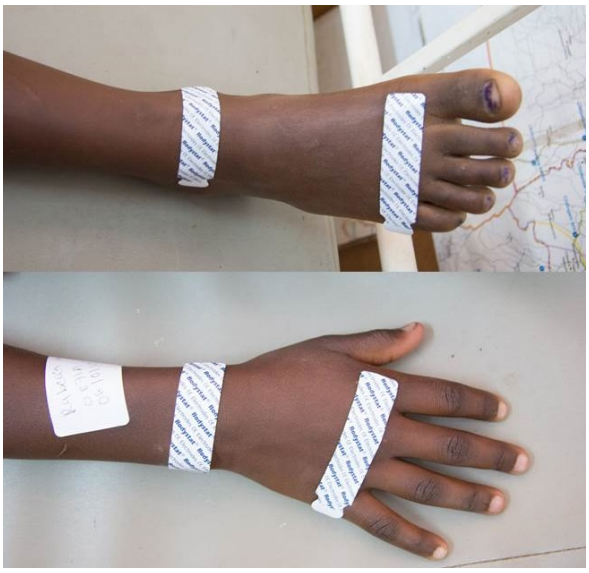


Image 9: Position of BIA electrode stickers on right hand and right foot

5.3.3 Skinfold Thickness

Skinfold thickness was measured using Holtain Tanner/Whitehouse Skinfold Callipers (Holtain Ltd, UK). Skinfold thickness was measured on 4 locations across the body: bicep, tricep, subscapular and suprailiac, on the left side of the body. The measurements were conducted independently by two operators, as with the other anthropometric measures. The callipers were set to zero before each measurement. The location of measurements was marked on the skin with eyeliner pencil, and the thumb and forefinger of the left hand were used to elevate a fold of skin and subcutaneous fat about 1cm away from the measurement point. The right hand was then used to place the callipers over the skinfold, perpendicular to the long axis of the fold, approximately half way between the crest of the fold and the body surface. The reading was taken once the dial of the callipers slowed to an almost stop. Measurements were recorded to the nearest 2mm.

Location of each skinfold measure:

- **Bicep skinfold**: a perpendicular line was drawn directly over the humerus and a horizontal line was draw at the same place as the MUAC reading (midway between the acromion process and the olecranon process), making a cross.
- **Tricep skinfold**: a similar cross was made at the back of the arm, also at the point of the MUAC reading and directly over the humerus.
- **Subscapular skinfold**: the inferior angle at the lower margin of the scapular was located and a diagonal line drawn around 1cm out from the shoulder blade corner. A perpendicular line to this one was drawn around 1cm from the scapular.
- **Suprailiac skinfold**: the operator located the top of the iliac crest (the same location as used for waist circumference) and marked this with a horizontal line. A cross was made by drawing a vertical line in line with the mid-axillar. The skinfold was grasped just above the cross, at a 45˚angle, following the natural cleavage of the skin.

Image 10: Marking location and measuring skinfold thickness for triceps and biceps

Image 11: Marking location and measuring suprailiac and subscapular skinfold thickness

5.4 Analysis

Primary analysis compared sibling and community controls to cases using linear regression analysis for:

- Relative waist circumference, relative hip circumference and calf circumference
- Tricep, bicep, subscapular and suprailiac skinfold thickness z scores
- Waist/hip ratio and skinfold thickness ratio
- Lean mass index and fat mass index proxy derived from BIA impedance readings
- R/h and Xc/h derived from BIVA

Potential confounding factors were adjusted for in multivariable linear regression; namely HIV status, age, sex, puberty status and SES, based on *a priori* assumptions. A conceptual framework of potential confounders is depicted in Figure 4b, Chapter 2.

Secondary analysis considered if any factors at admission predict poor body composition at 7-years post-discharge, namely severity of stunting and wasting at admission, age at admission and presence of oedema, as well as reported birth weight, for case children only. I also consider the impact of HIV and sex on body composition.

5.4.1 Data Cleaning

As with the growth data, I checked for erroneous values among the anthropometric measures by plotting scatter graphs of each variable against age and height. Outliers were identified and paper files checked for notes, corrections or explanations. Highly improbable readings without explanation were removed: 3 waist circumference readings and 1 hip circumference reading were removed. No skinfold thickness data was identified as anomalous though checking ranges, ratios and scatter plots against weight; this data has a large range making it difficult to identify erroneous entries. In order to clean BIA data, the first and second readings were compared, and any with a difference greater than 20ohms identified. 2 children had readings which were inconsistent and therefore, without being able to conclude which, if any, of the readings was correct, both sets of readings were removed from analysis. Scatter plots of BIA readings against weight identified no further major anomalies.

For analysis, an average of the first and second BIA readings was used.

5.4.2 Calculations and Statistics

For body composition, anthropometric measures were not only analysed in their raw state but also converted to ratios and relative measures detailed below:

- **Relative waist circumference** = waist circumference / height
- **Relative hip circumference** = hip circumference / height
- **Waist:hip ratio** = waist circumference / hip circumference
- **Skinfold thickness ratio** = (subscapular+waist) / tricep

Skinfold z scores using the LMS method

In the absence of relevant reference data for skinfold thickness, I used "LMSChartmaker" software to create an LMS reference table for males and females aged 4-15 years for each skinfold measure (bicep, tricep, subscapular and waist) using the data from sibling and community controls. The LMS method provides a way of obtaining normalized growth centile standards by summarising the changing distribution of a measure (e.g. skinfold thickness) according to a covariate (e.g. age). It assumes that the data can be normalized by using a power transformation, which stretches one tail of the distribution and shrinks the other, removing the skewness. Using data from sibling and community controls, the software calculates the optimal power to obtain normality for each age category and the trend is summarized by a smooth (L) curve. Trends in the mean (M) and coefficient of variation (S) are also smoothed. The resulting L, M and S curves contain the information to draw a centile curve which is used to generate a reference table with approximate standard errors for the smoothed centiles for each age category. I then used "LMSGrowth" software to calculate skinfold z scores for each child, including cases, by entering the child's age, sex and skinfold readings [222, 237] (http://www.healthforallchildren.com/lms-chartmaker-light-download/).

Lean mass index

In order to calculate a proxy for lean mass and total body water, a lean mass index equivalent (LMI) was calculated based on impedance index (H^2/Z) and height, as described by Wells et al 2007[229]. Lean mass index is lean mass accounting for height. A value proportional to LMI can be obtained by calculating impedance index and then dividing it by height^2. This calculation results in a value called 1/z; use of 1/z opposed to LMI avoids the need for population-specific conversion equations. 1/z is statistically the same as lean mass index but the units are abstract rather than in kilograms. The residuals of BMI regressed on 1/z provide a value proportional to fat mass index.

- **Impedance index = height^2 / impedance**
- **1/z (equivalent to LMI)= (height^2 / impedance) / height^2**

BIVA

Besides 1/z, I also used BIA data in BIA vector analysis (BIVA). Again, in order to avoid converting BIA readings into total body water (TBW) using equations which are not suitable for this population, BIVA can be used to consider the combined changes in resistance (R) and reactance (Xc) on the "*RXc"* mean graph, where the average of R/h and Xc/h are plotted as line segments with the 95% confidence ellipse[231]. This method allows for consideration of hydration as well as lean mass, which is not possible using the LMI method described above. It requires the following calculations:

- **R/h = resistance / height (m)**
- **Xc/h = reactance / height (m)**

I used "BIVA Software 2002" created by Piccoli & Pastori (Department of Medical and Surgical Sciences, University of Padova, Padova, Italy, 2002) to create my RXc mean graphs with ellipses. The software is an Excel file with Visual Basic macros; the means and standard deviations for R/h and Xc/h for each study group are entered into the software and it creates the graph[238] (http://www.renalgate.it/formule_calcolatori/BIVAguide.pdf).

5.5 Results

5.5.1 Missing Data

Anthropometry, BIA and skinfold thickness readings were available for almost the whole sample, with only a handful of missing readings for each variable. As already mentioned, a few readings were removed during data cleaning and other reasons for missing data included children refusing to cooperate or children not attending the hospital appointments, as with growth measures. For a full table of number of results for each outcome see **Table 5** in Chapter 3: Section 3.1.

5.5.2 BIA repeatability

Routinely, BIA readings were repeated for each child immediately after the first reading, in order to highlight and correct any obviously erroneous readings. To test the reliability of the BIA machine at different times of the day, I measured a subset of children 2 hours after their initial BIA reading and compared the two results. **Figure 21** shows scatter plots and Pearson's correlation coefficients for repeat readings of each of the four BIA outcomes. Using this method impedance had the highest correlation between repeat readings.

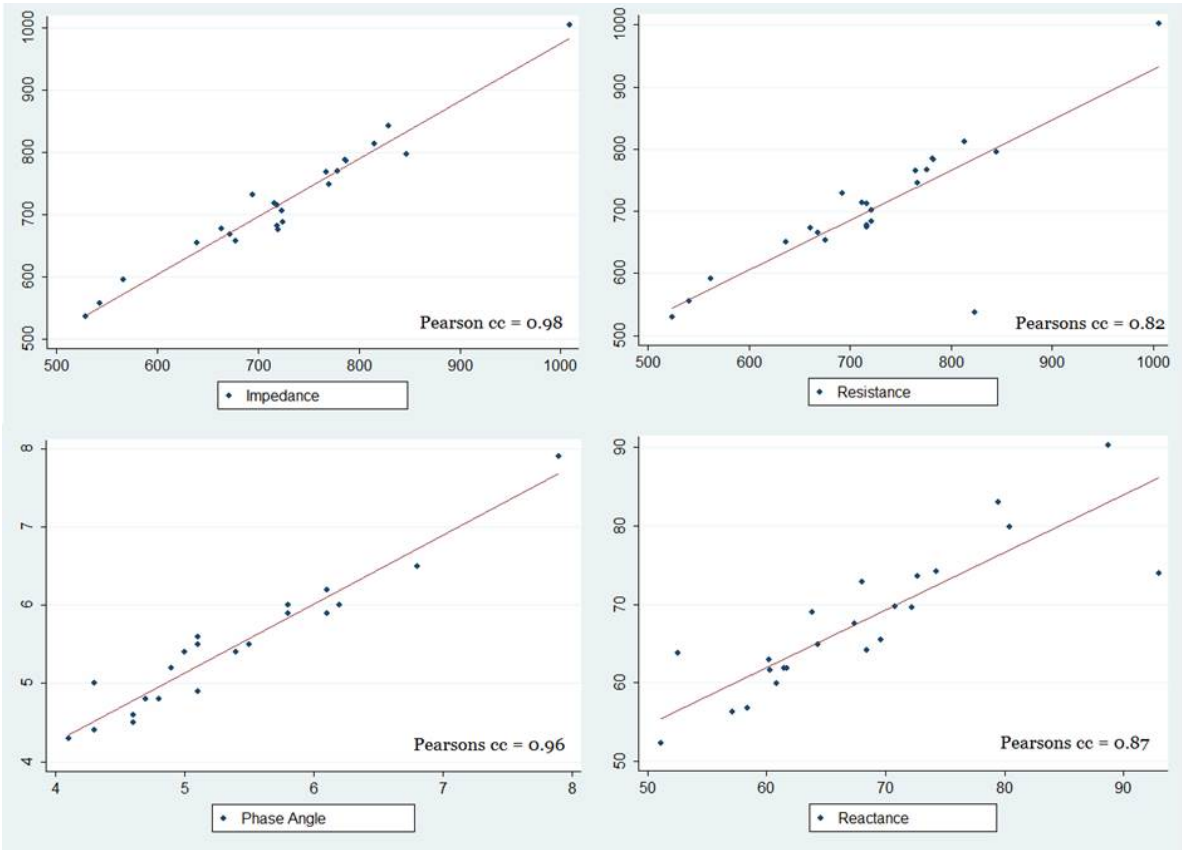


Figure 21: Scatter plots with regression line showing the correlation between repeat readings for each of the four BIA outcomes: Impedance, Resistance, Phase Angle and Reactance

Using a paired t-test (n=23) which is a comparison of the means, there was no significant difference in impedance, reactance, resistance or phase angle across the 2 readings. Using this method, reactance was the most robust of the readings with a *p* value of 0.96 and mean difference of just 0.06 ohms between repeats; see results in **Table 19**.

Measure (n=23)	Mean 1st reading	Mean 2nd Reading	Mean difference (SD)	95% CI	P value
Impedance	726.0	720.8	5.22 (22.39)	-4.5, 14.9	0.28
Resistance	722.9	704.5	18.43 (62.51)	-8.6, 45.5	0.17
Reactance	67.7	67.6	0.06 (5.28)	-2.2, 2.3	0.96
Phase Angle	5.3	5.4	-0.09 (0.24)	-0.19, 0.01	0.09

Table 19: Results of paired t-test comparing repeat BIA readings in order to assess BIA reading consistency. 2nd BIA reading was taken at least 2 hours after the first reading on a subset of children (n=23).

Besides correlation and regression, the Bland-Altman method is an alternative way for analysing repeatability[239]. It was proposed as an alternative to these methods as it analyses "agreement" between two readings rather than correlation or difference between the means. Bland-Altman graphs plot the difference between reading 1 and reading 2 on the Y axis against the mean of reading 1 and reading 2 on the X axis. This X axis (i.e. the means of the readings) represents the "true value". The mean of the differences should be zero for perfect "agreement". The mean of the differences is the same as the mean difference listed in **Table 19**. Bland and Altman state that 95% of data points should be within ±2 SD of the mean difference, as this is the "limit of agreement"[239]. This is true for all four BIA outcomes (Bland-Altman plots for impedance, resistance, reactance and phase angle can be seen in **Appendix 15**).

5.5.3 Body Composition of Controls vs Cases

5.5.3.1 Calf, Waist & Hip Circumferences and Skinfold Thickness

I hypothesised that case children would have less peripheral fat (and peripheral mass) than control children, and relatively more central fat than control children, based on a similar adaptation seen in low birth weight infants. **Table 20** shows means by study group for each anthropometric measurement, and the results of both simple and multivariable linear regression analysis comparing the groups. See **Appendix 16** for histograms of all outcomes in this chapter. Regression analysis shows that:

- Calf circumference is significantly smaller for cases than both sibling and community controls
- Cases have smaller relative hip circumference than sibling controls
- Cases have larger relative waists circumference than community controls
- Cases have larger waist/hip ratio than community controls due to the smaller hips of the cases

It is also worth noting that, as discussed in the previous chapter, MUAC is significantly smaller for cases than controls. These results indicate that cases have less peripheral mass than controls. MUAC and other circumferences cannot differentiate between fat and lean mass, however skinfold thickness specifically measures fat mass. When using skinfold thickness z scores to assess core and peripheral fat levels, the adjusted difference in peripheral fat is similar between the groups, except bicep skinfold is slightly larger for sibling controls than cases. Siblings also have significantly larger waist and subscapular skinfold thickness, suggesting that siblings have both more core and peripheral fat than cases, but skinfold thickness ratio is similar across all the groups.

When considering the HIV negative children only, the results are the same (see table in **Appendix 17**) except relative hip circumference which is not significantly different between cases and siblings when HIV positive children are removed from analysis. This is due to the HIV positive siblings having a significantly larger relative hip circumference than HIV negative siblings.

Anthropometry	Cases	Sibling			Community		
	Mean (SD)	**Mean** (SD)	**Unadjusted difference** (95% CI) *P value*	**Adjusted difference** (95% CI) *P value*	**Mean** (SD)	**Unadjusted difference** (95% CI) *P value*	**Adjusted difference** (95% CI) *P value*
Peripheral fat							
Calf circumference (cm)	23.7 (2.3)	25.0 (3.5)	1.36 * (0.9, 1.9) *<0.001*	0.62 * (0.2, 1.0) *0.002*	24.3 (2.4)	0.59 * (0.2, 1.0) *0.02*	0.49 * (0.1, 0.9) *0.02*
Hip circumference (cm)	62.3 (5.8)	65.9 (10.2)	3.51 * (2.1, 4.9) *<0.001*	1.83 * (0.8, 2.8) *<0.001*	63.7 (6.9)	1.34 (-0.2, 2.8) *0.08*	1.56 * (0.5, 2.7) *0.001*
Relative hip circumference	0.50 (0.0)	0.51 (0.0)	0.01 * (0.0, 0.0) *0.02*	0.01 * (0.0, 0.01) *0.04*	0.50 (0.0)	0.00 (-0.0, 0.0) *0.94*	0.00 (-0.0,0.0) *0.66*
Tricep skinfold thickness z score	-0.10 (1.1)	0.11 (0.9)	0.21 * (0.0, 0.4) *0.04*	0.16 (-0.0, 0.4) *0.12*	-0.14 (1.1)	-0.04 (-0.3,0.0) *0.67*	-0.07 (-0.3, 0.2) *0.63*
Bicep skinfold thickness z score	-0.2 (1.2)	0.06 (1.0)	0.26 * (0.1,0.5) *0.01*	0.23 * (0.0, 0.4) *0.04*	-0.07 (1.0)	0.13 (-0.1,0.4) *0.23*	0.14 (-0.1, 0.4) *0.22*
Core fat							
Waist circumference (cm)	56.3 (4.2)	57.7 (6.4)	1.45 * (0.5, 2.3) *0.002*	0.55 (-0.2, 1.3) *0.15*	56.0 (4.5)	-0.25 (-1.2, 0.7) *0.61*	0.08 (-0.7, 0.9) *0.86*
Relative waist circumference	0.45 (0.0)	0.45 (0.0)	-0.01 * (-0.01,-0.0) *0.045*	-0.00 (-0.0, 0.0) *0.73*	0.44 (0.0)	-0.01 * (-0.02, -0.0) *0.002*	-0.01 * (-0.1,-0.0) *0.01*
Waist skinfold thickness z score	-0.15 (1.0)	0.15 (1.0)	0.29 * (0.1,0.5) *0.002*	0.27 * (0.1, 0.5) *0.004*	-0.19 (1.0)	-0.04 (-0.2, 0.2) *0.67*	-0.05 (-0.3, 0.5) *0.69*
Subscapular skinfold thickness z score	-0.20 (1.1)	0.13 (1.0)	0.33 * (0.1, 0.5) *0.001*	0.30 * (0.1, 0.5) *0.004*	-0.17 (1.0)	0.03 (-0.2, 0.2) *0.76*	0.02 (-0.2, 0.2) *0.82*
Ratio of core to peripheral fat							
Skinfold thickness ratio	1.7 (0.4)	1.8 (0.4)	0.04 (-0.0, 0.1) *0.31*	0.03 (-0.1, 0.1) *0.53*	1.7 (0.4)	0.02 (-0.1, 0.1) *0.69*	0.03 (-0.1, 0.1) *0.50*
Waist/hip ratio	0.91 (0.1)	0.88 (0.1)	-0.02 * (-0.03, -0.0) *0.001*	-0.01 (-0.02, 0.0) *0.07*	0.89 (0.1)	-0.02 * (-0.03, -0.0) *0.006*	-0.02 * (-0.0,-0.0) *0.01*

Table 20: Means and results of simple and multivariable regression analysis for body composition anthropometry. Adjusted differences include HIV status, SES, puberty, age and sex in the linear regression model. Calf circumference adjusted difference also includes adjustment for presence of a physical disability. * indicates *p* value less than 0.05. Relative hip circumference= hip circumference/height; Relative waist circumference= waist circumference/height; Skinfold thickness ratio = (subscapular+waist) / tricep

5.5.3.2 BIA: Lean Mass Index

Table 21 shows mean values for each study group for 1/z (LMI equivalent) and BMI regression residual from 1/z (fat mass equivalent), as well as results of simple and multivariable linear regression analysis between the groups. There is no significant difference in lean mass or fat mass between cases and either type of control when assessed by this method. However, in the scatter plots of 1/z against age for the 3 study groups (**Figure 22**), it is clear to see that among the cases there are more children with very low LMI (<8) than in the control groups. When conducting analysis with HIV negative children only, results are similar except sibling controls have significantly more lean mass than cases once HIV positive children are removed from analysis (see results in **Appendix 17**).

	Cases	Sibling			Community		
	Mean (SD)	**Mean** (SD)	**Unadjusted difference** (95% CI) *P value*	**Adjusted difference** (95% CI) *P value*	**Mean** (SD)	**Unadjusted difference** (95% CI) *P value*	**Adjusted difference** (95% CI) *P value*
1/z (lean mass)	9.02 (1.1)	9.20 (1.0)	0.17 (-0.0,0.4) *0.08*	0.13 (-0.1,0.3) *0.19*	9.12 (1.0)	0.09 (-0.1, 0.3) *0.36*	0.04 (-0.2,0.3) *0.72*
BMI residual from 1/z (fat mass)	-0.15 (1.2)	0.28 (1.8)	0.43 * (0.2, 0.7) *0.002*	0.22 (-0.0, 0.5) *0.08*	-0.08 (1.6)	0.10 (-0.2, 0.4) *0.67*	0.15 (-0.1, 0.4) *0.27*

Table 21: Results of simple and multivariable linear regression analysis; adjusted difference includes HIV status, SES, puberty, age and sex in the regression model. * indicates $p<0.05$. z= impedance.

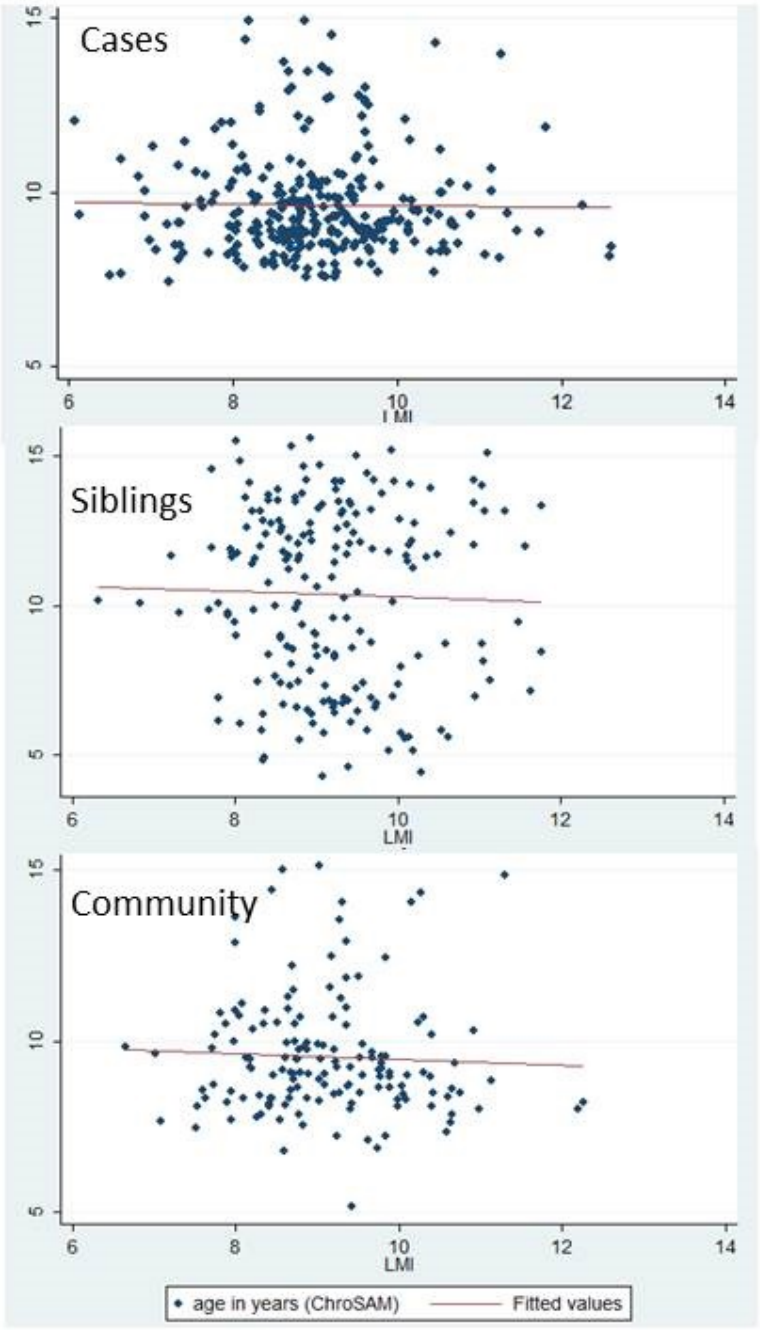


Figure 22: scatter plots of LMI equivalent (1/z) against age for cases, siblings and community controls

5.5.3.3 BIA Vector Analysis (BIVA)

The outcome of BIVA is graphical (**Figure 23)** and indicates that community controls have more lean mass, compared to cases and siblings. It also suggests that cases have less total body water than community controls, and even less compared to siblings. In order to adjust for potential confounders such as HIV, SES, age, gender and puberty, R/h, Xc/h and phase angle were analysed with linear regression. Result presented in **Table 22** show that there is a significant difference in R/h and phase angle between cases and community controls after adjusting for HIV, age, sex, SES and puberty. There is no difference in R/h, Xc/h and phase angle between cases and sibling controls after potential confounders are adjusted for. The reduced R/h of the siblings seen in the graph (Figure 22) can be largely explained by the difference in age between siblings and the other 2 groups; particularly the male siblings, who are significantly older than the cases and more hydrated than the other groups. BIVA suggests that cases have significantly less fluid and less lean mass than community controls, but similar levels to siblings. Cases have significantly smaller adjusted phase angle than community controls (p=0.004), which is associated with lower lean mass as well as nutritional and morbidity risk[235]. Results of adjusted analysis are the same when excluding HIV positive children from the model (see **Appendix 17**).

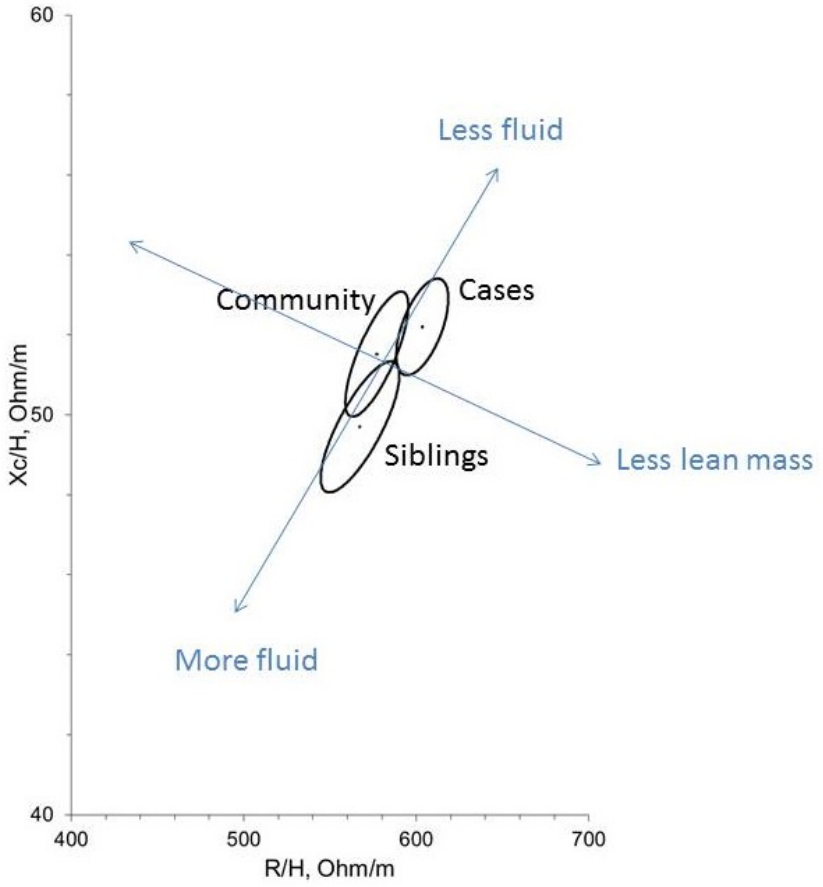


Figure 23: Results of BIVA for cases, siblings and community controls 7 years post-discharge

	Cases (n=294)	Sibling (n=198)			Community (n=151)		
	Mean (SD)	**Mean** (SD)	**Unadjusted difference** (95% CI) *P value*	**Adjusted difference** (95% CI) *P value*	**Mean** (SD)	**Unadjusted difference** (95% CI) *P value*	**Adjusted difference** (95% CI) *P value*
R/h	603.5 (105.1)	567.4 (129.6)	-36.1 * (-56.1,-16.1) *<0.001*	-11.5 (-29,5.8) *0.19*	577.1 (90.7)	-26.4 * (-48.1,-4.6) *0.02*	-24.5 * (-43,-5.5) *0.01*
Xc/h	52.2 (8.4)	49.7 (9.3)	-2.5 * (-4.1,-0.9) *0.002*	-0.5 (-2.1, 1.0) *0.52*	51.5 (7.8)	-0.7 (-2.3, 1.0) *0.43*	0.3 (-1.4, 1.9) *0.75*
Phase angle °	5.0 (0.7)	5.1 (0.7)	0.1 (-0.1,0.2) *0.09*	0.1 (-0.0, 0.2) *0.16*	5.1 (0.5)	0.1 (-0.0,0.2) *0.07*	0.2 * (0.1, 0.3) *0.003*

Table 22: Results of simple and multivariable linear regression analysis; adjusted difference includes HIV status, socioeconomic status, puberty, age and sex in the regression model. * indicates $p<0.05$. R/h = resistance/height(m); Xc/h=reactance/height(m)

5.5.4 Impact of HIV on Body composition

HIV is not associated with significant differences in body composition at this age, in contrast to its impact on linear growth. **Table 23** presents means and standard deviation values for main body composition outcomes stratified by HIV status and study group. Note the small numbers of HIV positive children in the control groups. For cases only, regressing body composition outcomes by HIV status shows no significant difference between HIV positive and HIV negatives for calf circumference, skinfold thickness ratio, hip circumference, waist/hip ratio, LMI equivalent, fat mass equivalent and R/h. HIV positive children have a significantly higher Xc/h (coef. 2.80, 95% CI 0.7 to 4.9, p=0.01) and phase angle (coef 0.18, 95% CI 0.0 to 0.5, p=0.045). This is unexpected, as it suggested that HIV positive case children have healthier cell membranes than HIV negative children and lower morbidity risk, however there is some evidence that ARV therapy can improve BIA outcomes and as all these children are controlling their HIV with medication, perhaps this explains their slightly improved body composition[240].

	Cases		Sibling Controls		Community Controls	
	HIV positive	HIV negative	HIV positive	HIV negative	HIV positive	HIV negative
N	89	198	9	125	4	92
Calf circumference	23.6 (2.1)	23.6 (2.3)	23.0 (2.2)	25.1 (3.7)	23.2 (1.2)	24.1 (2.3)
Skinfold ratio	1.77 (0.4)	1.69 (0.4)	1.62 (0.4)	1.73 (0.4)	2.01 (0.4)	1.76 (0.4)
Hip circumference	62.1 (5.8)	62.5 (5.7)	64.5 (14.2)	66.0 (11)	61.4 (4.4)	63.5 (7.1)
Waist/hip ratio	0.91 (0.1)	0.90 (0.1)	0.89 (0.1)	0.88 (0.1)	0.92 (0.1)	0.89 (0.1)
1/z (lean mass)	8.9 (1.0)	9.1 (1.1)	8.7 (0.7)	9.2 (0.9)	8.9 (0.8)	9.1 (1.0)
BMI residual (fat mass)	-0.02 (1.2)	-0.20 (1.2)	0.16 (0.9)	0.34 (2.0)	0.03 (1.7)	-0.10 (1.8)
R/h	615 (118)	601 (101)	666 (123)	569 (130)	580 (35)	580 (97)
Xc/h	54.3 (9.3)	51.5 (7.9)	58.9 (9.3)	49.8 (9.7)	51.4 (4.9)	52.0 (7.4)
Phase angle	5.12 (0.7)	4.94 (0.67)	5.09 (0.40)	5.09 (0.7)	5.05 (0.48)	5.14 (0.6)

Table 23: Means and standard deviations for body composition outcomes stratified by HIV status and study group are presented. Those with unknown HIV status are not included. Skinfold thickness ratio= (subscapular+waist) / tricep.

5.5.5 Body composition and Sex

There are significant differences in body composition between sexes which are worth highlighting. These differences are consistent across the study groups. After adjusting for case/control status, HIV and age, there is no significant difference in skinfold thickness ratio or phase angle between girls and boys. However, boys have significantly smaller calves than girls (coef -0.57, 95% CI -0.8 to -0.2, p<0.001) and smaller hip circumferences (coef -1.85, 95% CI -2.7 to -1.0, p<0.001) and boys have significantly larger waist/hip ratio than girls (coef 0.03, 95% CI 0.02 to 0.04, p<0.001). Boys also have more lean mass (1/z) than girls (coef 0.44, 95% CI 0.3 to 0.6, p<0.001) and less fat mass (coef -0.30, 95% CI -0.5 to -0.1, p<0.001). Boys have significantly lower R/h (coef. -17.0 p=0.02) and lower Xc/h (coef. -1.88, p<0.001) than girls. Considering these large sex differences, I conducted a stratified analysis of cases vs controls for boys and for girls and found that the differences in body composition between cases and controls are more prominent for girls than for boys (result of sex stratified regression analysis can be seen in **Appendix 18**). Waist circumference, waist/hip ratio and phase angle are not significantly different between community control boys and case boys, whereas girls are. Conversely, only community control boys have significantly lower R/h than cases, girls do not have significantly different R/h.

5.5.6 Predictors of adverse Body composition in cases

Lastly, I explored factors at admission which might predict adverse body composition outcomes at 7 years post-discharge. **Table 24** presents raw data (means (SD)) for some of the main body composition outcomes of children with different characteristics at admission. Further analysis shows that low WAZ and HAZ at admission and marasmus rather than kwashiorkor are associated with adverse body composition at 7 years post-discharge. Age at admission and reported birth size are not associated with body composition outcomes.

Mean (SD)	Calf circumferen	Waist/hip ratio	1/z (LMI)	R/h	Phase angle
Degree of wasting at admission					
Low WAZ <-4 (n=137)	22.9 (2.2)	0.93 (0.1)	8.90 (1.1)	634 (114)	4.92 (0.8)
High WAZ >-4 (n=160)	24.3 (2.1)	0.89 (0.1)	9.13 (1.0)	579 (89.1)	5.05 (0.6)
Degree of stunting at admission					
Low HAZ (<-3) (n=143)	23.2 (2.2)	0.92 (0.1)	9.06 (1.1)	618 (98.7)	4.93 (0.7)
High HAZ (>-3) (n=157)	24.1 (2.3)	0.89 (0.1)	8.98 (1.1)	592 (110)	5.05 (0.7)
Kwashiorkor or wasting					
Oedema (n=265)	23.8 (2.2)	0.90 (0.1)	9.09 (1.0)	594 (94.5)	5.00 (0.6)
No oedema (n=54)	22.9 (2.4)	0.92 (0.1)	8.76 (1.2)	649 (138)	4.95 (0.8)
Age at admission					
<2 years (n=158)	23.1 (2.0)	0.92 (0.1)	9.03 (1.1)	623 (108)	4.89 (0.6)
>2 years (n=145)	24.3 (2.4)	0.90 (0.1)	9.03 (1.1)	583 (98.4)	5.11 (0.7)
Reported birth size					
Small at birth (n=24)	22.7 (1.7)	0.90 (0.1)	8.74 (1.1)	635 (82.5)	5.10 (0.5)
Normal size at birth (n=270)	23.8 (2.3)	0.91 (0.1)	9.07 (1.1)	600 (108)	4.99 (0.7)

Table 24: Means and standard deviations for main body composition outcomes stratified by characteristics at admission

Degree of Wasting and Stunting at admission

Those with greater stunting or greater wasting at admission have significantly smaller calf circumference and phase angle at 7 years post-discharge. They also have significantly larger waist/hip ratio and significantly larger R/h at 7 years post-discharge. Those who had a WAZ larger than -4 at admission do not have significantly different calf circumference, waist/hip ratio, lean mass equivalent, R/h and phase angle to control children.

Kwashiorkor at admission

When considering those cases who were admitted with and without oedema 7 years ago, we can see that survivors who were admitted without oedema (i.e. marasmus) have a significantly higher R/h than controls at 7 years post-discharge, whereas those survivors admitted with oedema have similar R/h to controls. This suggests that those who were admitted with marasmus now have significantly less fluid and less lean mass than controls, whereas those who were admitted with kwashiorkor have similar lean mass and hydration to controls at 7 years post discharge (see **Figure 24**). Additionally, calf circumference is significantly lower in marasmus survivors than kwashiorkor survivors and lean mass equivalent (1/z) is lower in marasmus survivors than kwashiorkor survivors with borderline significance (p=0.08). Waist/hip ratio and phase angle are not significantly different between marasmic and kwashiorkor survivors.

The body composition differences seen between cases and controls is more pronounced in survivors who had marasmus; those who had kwashiorkor seem to have retained a more "normal" body composition at 7 years post discharge. This correlates with results in previous chapter which showed marasmic survivors to have significantly lower WAZ at 7 years post-discharge than kwashiorkor survivors.

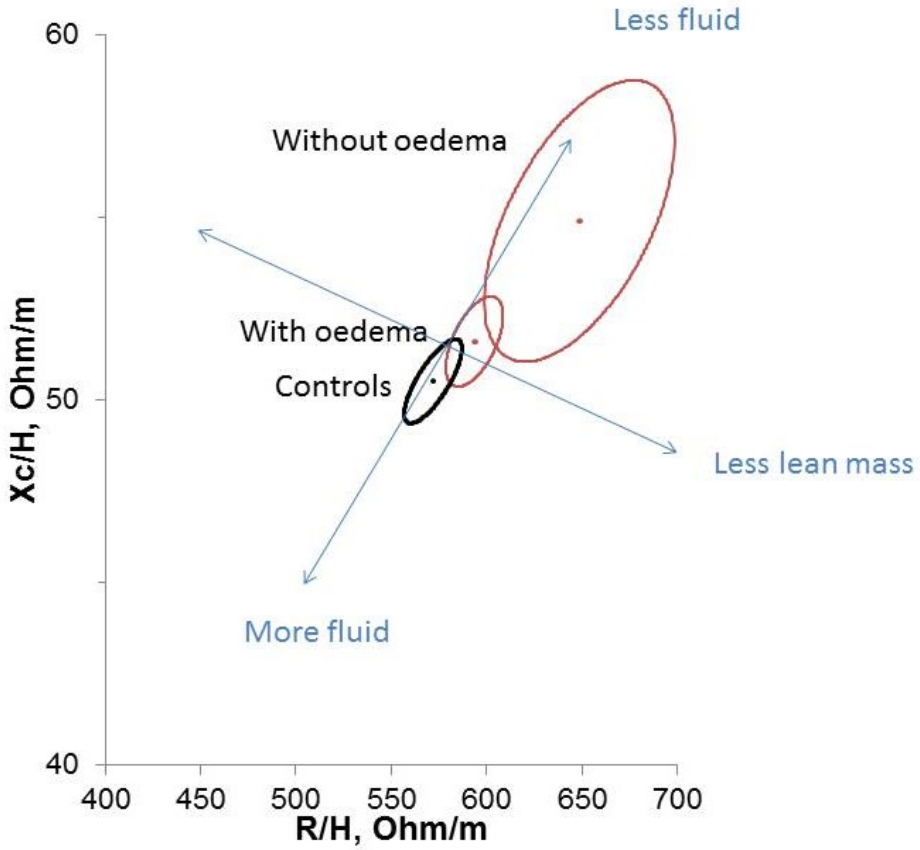


Figure 24: BIVA results for those children who had oedema at admission, and those that didn't, and community controls, at 7 years post-discharge.

5.5.7 Summary

This chapter explored various aspects of body composition including body circumferences, skinfold thickness, and BIA. Results show that SAM-survivors do have some elements of altered body composition compared to controls. Cases have smaller calf circumference to controls; they also have smaller or same sized relative hip circumference to controls, and larger or same sized relative waists to controls. This suggests an unhealthy ratio of core to peripheral mass with cases having less peripheral lean mass. However, when it comes to subcutaneous fat, skinfold thickness results suggest that although siblings have significantly more subcutaneous fat, the ratio of core to peripheral subcutaneous fat is the same as cases. Additionally, when assessing overall fat mass using BIA-BMI regression residuals, there is no significant difference between cases and control. LMI equivalent as calculated from BIA also shows no difference between cases and controls, however when the impedance reading is broken down into resistance and reactance using BIVA we find that cases have significantly less fluid and less lean mass than community controls, but similar levels to siblings. Cases also have lower phase angle than community controls which is associated with nutritional and morbidity risk. These effects are particularly pronounced in females at this age, and in those children who were admitted with wasting rather than those with kwashiorkor. Adverse body composition is also more evident in those admitted with a greater degree of wasting or stunting 7 years ago.

6 Chapter 6: Physical Function

This chapter discusses physical function and the potential effects that SAM could have on long-term physical function. Besides anthropometry and body composition, discussed in previous chapters, it is important to explore whether clinical insults are having any impact on everyday function such as physical function. Specific areas of physical function discussed here are muscle strength, daily physical activity and physical capacity for exercise. This chapter also describes the specific methods, analysis and results related to physical function outcomes; primarily comparing physical function of cases to controls to assess whether an episode of SAM has had enduring detrimental effects in this area. Additionally, I present results exploring the links between body composition and physical function, and exploring variables among cases at admission that may predict poor physical function at 7 years post-discharge.

6.1 Chapter-specific Hypotheses

Physical function is the first of my functional outcomes to be explored. For the purpose of this study physical function refers to muscle strength and physical fitness, as these are the areas I felt were most relevant to SAM-survivors, as well as having important implications for chronic diseases in adulthood and major implications for productivity and earning-potential[96, 138]. The long-term implications that SAM has had on growth and body composition may well translate to important detrimental impacts on physical function. I hypothesised that:

4. Compared to controls, SAM survivors have (a) lower levels of physical activity, (b) lower physical capacity during an exercise test and (c) weaker hand grip strength.

6.2 Specific Methods

6.2.1 Hand Grip Strength

Hand grip strength was measured using a "Takei Grip-D" dynamometer device (Japan) which measures hand grip strength from 5kg and above. The child held the device in their hand, with their elbow by their side and at right angle. The operator often needed to hold the other side of the device to steady it. The child then squeezed the device with as much effort as possible and with no other movement of their body. Three attempts were made with each hand and any disabilities which may interfere with the reading were noted.

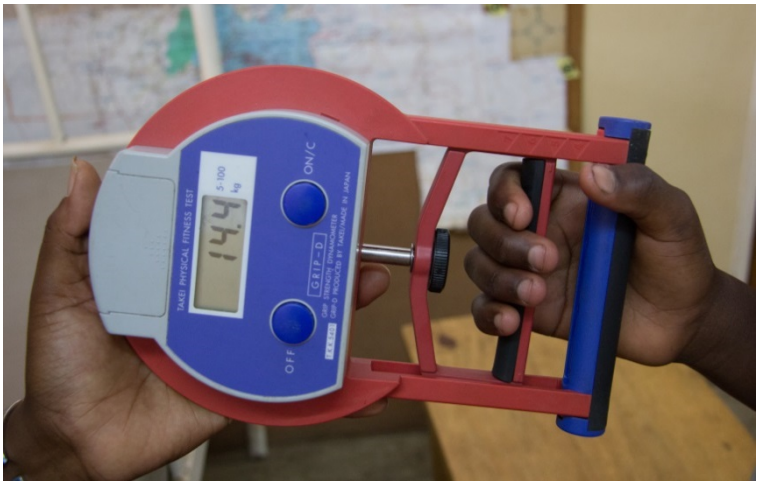


Image 12: Takei Grip-D device used to measure hand grip strength in kg

6.2.2 Physical Activity: Accelerometers

In order to measure physical activity, the children were required to wear "ActiLife" accelerometers (Actigraph Corp, USA) around their waist for at least 48 hours. The device was fitted during the first visit to the child's home, and they were asked to wear the device at all times, including when sleeping. The child was asked to attend the hospital appointment wearing the device, at which point it was removed and the data downloaded.

Image 13: Three "ActiLife" accelerometers with elastic tape to fit around the children's waists

6.2.3 Physical Capacity: iStep Exercise Test

The iStep Test is a 10 minute exercise test to assess exercise capacity. The test was developed at Great Ormond Street Hospital (GOSH) with the UCL Institute of Child Health (UK) as part of a study looking at the development and validation of a "field-friendly" exercise test for children with Cystic Fibrosis[151]. It is an externally paced test on a single standard exercise step for children aged 6-16 years. The step height should be appropriate for the height of the child therefore we had 3 available steps of 15, 20 and 25cm. In order to select the correct step height, the child's lower limb was measured from ankle bone to the knee joint; the length was then divided by 2 and rounded down to the closest step height i.e. 33cm lower limb measurement ÷ 2= 16.5cm → therefore the 15cm step would be used.

The child was instructed to step up and down in time to the beeps. There are 5 levels to the test; each level is 2 minutes long; the test is a total of 10 minutes. The pace of the beeps becomes quicker at each level. The child was told that the aim should be to complete the test however if there was any point where they felt unwell or not able to carry on, they should stop. The following measurements were taken at baseline, during the test, at the end of the test and at one minute after the test:

- Heart Rate (HR)
- Oxygen saturation
- OMNI scale (for perceived exhaustion)[241]
- VAS (visual analogue scale for perceived breathlessness)

A NONIN PalmSAT 2500 pulse oximeter device (Nonin Medical Inc., USA) was used for measuring heart rate and oxygen saturation during the test as it has technology which allows for participant movement during measurement. Images of the English and Chichewa versions of OMNI and VAS scales can be seen in **Appendix 19**.

The main outcomes used in analysis were: highest HR, lowest oxygen saturation, time completed of the test, highest HR adjusted for stopping time (HR:Level ratio), drop in HR at recovery (as % of highest HR) and final HR in all those who completed the test.

Image 14: iStep exercise test in progress (top photo) followed by 1 minute recovery (bottom photo)

6.2.4 Sample Size Calculations

Due to lack of previous studies with these specific outcomes, it was difficult to calculate required sample sizes for most physical function measures. I did find a study for AMA and for hand grip strength that compared cases and controls with some relevance to nutrition. Based on our actual sample size and using evidence from these studies, I calculated the power of our sample size using Stata "*sampsi*" command. Benefice et al., (1992) found mean AMA of 21.8 (±2.8) in normal children compared to 18.7 (±2.9) in children with low WAZ; with AMA outcomes for 279 cases, 185 siblings and 140 community controls, our analysis is powered to 100% to detect this difference[142].

For hand grip strength, a study found a change in grip strength of 2.79kg (±3.11) following nutrition supplementation in adult TB patients[242]. Again, our existing sample size is powered to 100% to detect this difference. However this difference is perhaps larger than I would expect in this study as these are adults with a current infection being compared during recovery. Another study in adults suggested that a change of more than 6kg is required to be clinically significant in adults; the standard deviation was not provided[243]. I could not find a study with the equivalent value for children.

6.3 Analysis

Primary analysis compared sibling and community controls to cases using regression analysis for:

- Prevalence of physical disabilities (seeing, hearing, walking)
- Hand grip strength
- Arm-muscle area (AMA)
- Daily physical activity levels (average steps taken per hour)
- Physical capacity using iStep test

Logistic regression was used to analyse differences in binary outcomes for physical disabilities between the three study groups; both unadjusted odds ratio (OR) and adjusted OR at presented. Adjusted OR includes age, sex, HIV status and SES in the model as potential confounders.

For continuous outcomes (hand grip strength, majority of iStep outcomes and physical activity levels), simple and multivariable linear regression analysis was used to compare cases and both types of control. Potential confounding factors were adjusted for in multivariable linear regression; namely HIV status, age, sex, physical disability and SES, based on *a priori* assumptions. A conceptual framework of potential confounders is depicted in Figure 4c, Chapter 2. Ordered logistic regression was used to compare time completed of the iStep test as it is a categorical ordinal variable and it is not normally distributed.

Secondary analysis considered if any factors at admission predict poor physical function 7 years post-discharge, namely severity of stunting and wasting at admission, age at admission and presence of oedema, as well as reported birth weight, for case children only. I also considered the impact of HIV on physical function and explore to what extent differences in body size and composition (WAZ, HAZ and lean mass) predict physical function.

6.3.1 Calculations

As hand grip strength was measured on each hand 3 times, the best reading from either hand was used in analysis. AMA was calculated based on the formula proposed by Gurney & Jelliffe as follows[244]:

$$\textbf{AMA (cm}^2\textbf{)} = [\textbf{MUAC} - ((\textbf{Tricep skinfold} * \pi)]^2 / (4 * \pi))/100$$

Accelerometer data can be analysed in a number of ways; one options is to analyse proportion of the day spend sedentary or active, however definition of sedentary and active is based on standard cut-off points which may not be applicable to Malawian children, hence I decided not to use this and to simply use number of steps per hour as my variable for comparing physical activity levels between the groups. Steps per hour were calculated as follows:

Steps per hour = (total steps record / total minutes wear time) * 60

Four outcomes were derived from the iStep exercise test with the intention of assessing physical exercise capacity. Lowest oxygen saturation during the test and amount of time completed of the test, which require no calculations. The other two outcomes, heart rate/level ratio and percent drop in heart rate at 1 minute recovery, were calculated as follows:

Heart rate: Level ratio = highest heart rate / highest test level completed

Drop in HR at 1 minute recovery (%) = (heart rate at recovery / highest heart rate) *100

6.4 Results

6.4.1 Missing Data & Data Quality

Almost all of the sample have hand grip strength readings (298 cases, 200 siblings and 154 controls) and iStep test readings; the reason for a few missing results is either physical disability or illness (such as malaria) at the time of data collection.

Unfortunately, there is a lot of missing accelerometer data. There are a number of reasons for this. Firstly, not all children were asked to wear accelerometers largely due to the logistics of charging them at a computer. When I was not present in Malawi, the data team could not charge and initiate accelerometers as only 1 person can have access to the software and charging hub. Secondly, as hospital appointments were made at the family's convenience, accelerometers were only offered to those with a gap of at least 48 hours between home and hospital visits. Additionally, of those who were asked to wear accelerometers, some refused as they didn't like the look of them; most often this was due to the accelerometers being red in colour which is associated with blood and witchcraft in Malawi hence carrying negative associations in some communities. Others explained that they didn't want everyone to see their child wearing them, or they just didn't understand what they did and therefore were wary of them. Of the 156 children who were offered accelerometers and agreed to wear them, 121 actually wore them for at least a short time after the study team left. Of these 121, 78 wore them for more than 500 minutes in a day for 2 days and therefore had enough data for analysis.

For a full table of number of results for each outcome variable see **Table 4** in Chapter 3: Section 3.1.

6.4.2 Physical function of Controls vs Cases

6.4.2.1 Physical Disability

Table 25 shows results of logistic regression analysis for hearing, sight and walking disabilities, comparing the 2 control groups to the cases. Once adjusted for potential confounders, there is no significant difference in odds of having difficulty seeing between cases and controls; cases are more likely to have difficulty hearing than siblings. OR of having difficulty walking is significantly lower for community controls than cases, but not different for sibling controls. There could be an element of selection bias here as mothers with disabled children in the community control group may be less likely to agree to attend a hospital appointment due to the added logistical difficulties. Additionally, when searching for a community control in the village, families who were approached who have a child with a disability may have assumed that he/she was not eligible and therefore not volunteered that child.

	Cases (n= 313)	**Sibling** (n=209)			**Community** (n=175)		
	%	**%**	**Unadjusted OR** (95% CI) *P value*	**Adjusted OR** (95% CI) *P value*	**%**	**Unadjusted OR** (95% CI) *P value*	**Adjusted OR** (95% CI) *P value*
Difficulty seeing	0.06	0.04	-0.54 (-1.4, 0.3) *0.21*	-0.45 (-1.4, 0.5) *0.35*	0.06	-0.12 (-0.9, 0.7) *0.77*	0.16 (-0.7, 1.1) *0.73*
Difficulty hearing	0.17	0.07	-1.02 * (-1.6, -0.4) *<0.01*	-0.84 * (-1.5,-0.2) *0.02*	0.13	-0.33 (-0.9, 0.2) *0.23*	-0.08 (-0.7, 0.6) *0.81*
Difficulty walking	0.04	0.02	-0.79 (-1.9, 0.3) *0.17*	-1.20 (-2.5, 0.1) *0.08*	0.01	-2.02 (-4.1, 0.0) *0.05*	-2.20 * (-4.4, -0.0) *0.049*

Table 25: Percentage of each study group with a physical disability, and odds ratios (OR) comparing controls to cases generated using logistic regression analysis. Adjusted OR includes age, sex, HIV status and SES as potential confounders in the regression model. * indicates *p* value <0.05

6.4.2.2 Hand grip strength, Physical Activity & Physical Capacity

I hypothesised that, compared to controls, SAM survivors would have lower levels of physical activity, lower physical capacity during an exercise test and weaker hand grip strength. The mean values for each outcome as well as results of simple and multivariable regression analysis comparing hand grip strength, physical activity levels and physical capacity between controls and cases are presented in **Table 26**. Histograms for each outcome can be found in **Appendix 20**.

Average steps per day for the sample as a whole was 14,667 based on children wearing the device for more than 11 hours per day. Results show that cases take on average 239 fewer steps per hour than their sibling controls and 54 fewer steps per hour than community controls; however results of the regression analysis show that these differences did not reach the cut-off for significance. Post hoc sample size calculations show that the test is currently only powered to 18% and to achieve 90% power, based on the current means of the cases and community controls, sample size would need to be around 320 per group. However the difference between the cases and community controls is 0.13 standard deviations and difference between cases and siblings is 0.58 standard deviations, which is large in biological terms. This suggests there may be a difference in physical activity levels between ex-SAM cases and controls. When comparing HIV negative children only, siblings take significantly more steps than cases in both unadjusted and adjusted analyses (see table of result for HIV negative children only in **Appendix 21**). Age was not significantly associated with physical activity levels.

Community controls have significantly stronger hand grip strength than cases. Siblings also have stronger hand grip strength than cases using simple linear regression; after adjusting for age differences and other potential confounders (sex, HIV status and SES), the difference is decreased but still significance (p=0.005). When comparing HIV negative children only, sibling and community controls have significantly stronger hand grip strength than cases, using both simple and multivariable regression models (**Appendix 21**). Community controls and siblings also have significantly larger AMA than cases.

Physical capacity results based on outcomes from the iStep exercise test show some adjusted significant differences between cases and community controls. A larger proportion of sibling and community controls completed the exercise test than cases and community controls have 0.47 higher log-odds than cases of completing more minutes of the exercise test with marginal significance after adjusting for potential confounders (p=0.07) (**Table 27**). Community controls are therefore marginally less likely to opt out of the test before the end. In addition, despite completing few minutes of the exercise test, there is no significant difference in highest heart rate between the groups (Table 25). This suggests that the HR of community controls did not rise as much as cases, relative to the amount of exercise they completed. Lastly, community controls

have a significantly greater drop in HR during 1st minute of recovery than cases, indicating that their HR recovers more quickly than cases. This is presented as a percentage of their highest HR. For sibling controls, there is no adjusted significant difference in physical capacity outcomes, however the result are trending in the same direction as those of community controls. Neither siblings nor community controls have a significantly different "lowest oxygen saturation" than cases, indicating that oxygen diffusion during exercise is similar across the groups. This suggests that lung function in terms of oxygen diffusion has been unaffected by SAM; further lung function results are presented in Chapter 7. There was no difference in "perceived tiredness" or "perceived breathlessness" between cases and controls, nor in the final heart rate when comparing only those who completed the test. All the physical capacity results remain true when comparing HIV negative children only (see table in **Appendix 21**).

	Cases	Sibling			Community		
	Mean (SD)	**Mean** (SD)	**Unadjusted difference** (95% CI) *P value*	**Adjusted difference** (95% CI) *P value*	**Mean** (SD)	**Unadjusted difference** (95% CI) *P value*	**Adjusted difference** (95% CI) *P value*
Muscle Strength							
Hand grip strength (kg) n= 652	12.7 (6.3)	14.8 (7.9)	2.05 * (1.2, 2.9) *<0.001*	1.01 * (0.3, 1.7) *0.005*	13.8 (3.9)	1.39 * (0.4, 2.4) *0.005*	1.68 * (0.9, 2.4) *<0.001*
AMA (cm^2)	18.9 (4.5)	21.1 (6.3)	2.15 * (1.2, 3.1) *<0.001*	1.07 * (0.3, 1.8) *0.007*	20.1 (4.6)	1.21 * (0.6, 2.3) *0.02*	1.41 * (0.6, 2.3) *0.001*
Physical Activity							
Steps per hour n=78	859.8 (280)	1001 (298)	141.5 (-10.1, 293) *0.07*	144.5 (-37, 325) *0.12*	884.5 (224)	24.6 (-116, 166) *0.73*	-6.72 (-185, 172) *0.94*
Physical Capacity (iStep outcomes, n=778)							
Baseline HR	99.9 (15.1)	98.6 (15.4)	-1.29 (-4.5,1.9) *0.43*	-0.23 (-3.6,3.2) *0.89*	99.1 (14.4)	-0.76 (-4.1,2.6) *0.66*	-1.0 (-4.6,2.5) *0.57*
Highest HR	154.8 (21.9)	156.7 (20.9)	1.82 (-2.7,6.3) *0.43*	-0.47 (-5.3,4.4) *0.85*	159.5 (19.5)	4.22 (-0.5,9.0) *0.08*	4.24 (-0.9,9.3) *0.10*
HR:Level Ratio ~log	52.2 (35.2)	45.6 (28.9)	-0.10 (-0.2, 0.0) *0.06*	-0.08 (-0.2, 0.0) *0.19*	45.7 (27.3)	-0.09 (-0.2, 0.0) *0.10*	-0.11 (-0.2, 0.0) *0.07*
Lowest O_2 saturation	93.4 (4.9)	93.7 (4.9)	0.37 (-0.6, 1.4) *0.47*	0.73 (-0.4, 1.8) *0.19*	93.8 (3.8)	0.45 (-0.6, 1.5) *0.40*	0.29 (-0.9, 1.4) *0.63*
HR at 1 min recovery	113.6 (18.6)	112.9 (19.1)	-0.73 (-4.9,3.4) *0.73*	0.74 (-3.8, 5.3) *0.75*	110.9 (18.1)	-2.70 (-7.1,1.7) *0.23*	-1.35 (-6.1, 3.4) *0.58*
Drop in HR at recovery (as % of highest HR)	72.6 (10.8)	70.9 (10.9)	-1.71 (-4.1, 0.6) *0.15*	-0.24 (-2.8, 2.4) *0.86*	68.6 (10.0)	-3.95 * (-6.5, -1.4) *0.002*	-3.37 * (-6.1, -0.7) *0.01*
Final HR (if test was completed)	157.5 (18.6) n=125	157.6 (18.1) n=90	0.09 (-4.9, 5.1) *0.97*	0.87 (-4.9, 6.7) *0.77*	153.8 (17.8) n=78	-3.70 (-8.9, 1.5) *0.16*	-2.19 (-8.0, 3.6) *0.46*

Table 26: Mean (SD) and results of simple and multivariable linear regression analysis for hand grip strength and steps per hour for controls vs cases. Adjusted difference includes age, sex, HIV status and SES as potential confounders in the regression model. * indicates significant difference i.e. p value <0.05. HR = Heart Rate. ~ indicates that natural log of the raw value is being used

	Cases	Siblings			Community		
	% completed test (10 mins)	% completed test (10 mins)	Unadjusted log-odds (95% CI) *P value*	Adjusted log-odds (95% CI) *P value*	% completed test (10 mins)	Unadjusted log-odds (95% CI) *P value*	Adjusted log-odds (95% CI) *P value*
Time completed of iStep	59% (124/210)	69% (92/134)	0.47 * (0.0, 0.9) *0.04*	0.27 (-0.2, 0.8) *0.29*	68% (77/113)	0.47 (-0.0, 0.9) *0.05*	0.47 (-0.0, 1.0) *0.07*

Table 27: Result of ordered logistic regression comparing time completed of the iStep test for siblings and community controls with cases. Adjusted difference included age, sex, HIV status and SES in the regression model.

6.4.3 HIV and Physical Function

Interestingly, after adjusting for case/control status, HIV has no significant impact on hand grip strength, AMA, physical activity or physical capacity (time completed of iStep test).

6.4.4 Effect of Body Size and Composition on Physical Function

6.4.4.1 Body Size: WAZ and HAZ

We know from the previous section that cases have significantly weaker hand grip, smaller AMA and marginally lower physical capacity than community controls, is this only because they are shorter and lighter than controls?

Firstly, is there an association between weight/height and physical function? **Table 28** shows that as WAZ and HAZ increase, hand grip strength also increases significantly. Equally, AMA increases very slightly but significantly with increase in WAZ, HAZ and BAZ. However, physical capacity (in the form of time completed of the iStep test) is not associated with WAZ, HAZ or BAZ. Results for steps per hour have not been presented as sample size is too small, especially for stratification

	Hand grip strength (kg) **Unadjusted difference (95% CI)**	**AMA (cm^2)** **Unadjusted difference (95% CI)**	**Time completed of iStep (minutes)** **Unadjusted log-odds (95% CI)**
WAZ	0.10 * (0.07, 0.12)	0.001* (0.00, 0.01)	0.13 (-0.1, 0.4)
HAZ	0.05 * (0.03, 0.07)	0.001 * (0.00, 0.01)	0.12 (-0.0, 0.3)
BAZ	0.01 (-0.00, 0.02)	0.001 * (0.00, 0.01)	0.61 (-0.1, 0.3)

Table 28: Results of regression analysis of functional outcomes against anthropometric indicators for the cohort as a whole. * indicates significant difference i.e. *p* value <0.05

Distinguishing between the effects of a previous episode of SAM and the effects of a child's current WAZ and HAZ is very difficult, as they are very much interlinked. Age is also a potential confounder as it is associated with both WAZ/HAZ and physical function outcomes (older children tend to have lower WAZ and HAZ). However, if I compare cases who have HAZ >-1 (n=153) to controls who also have HAZ >-1 (n=167) whilst adjusting for age, we see no significant difference in physical capacity, but non-stunted cases still have significantly lower hand grip strength (coef -1.1, p=0.04) and AMA (coef -158, p=0.02) than non-stunted controls. If I consider cases with WAZ >-1.5 (n=96) compared to controls with WAZ >-1.5 (n=117) adjusting for age, I find no significant difference in hand grip strength, AMA or physical capacity. These results suggest that weight, specifically continued low weight in cases, is playing a large role in the difference in physical function between cases and controls. See **Figure 25** for boxplots of AMA and grip strength stratified by study group and WAZ category.

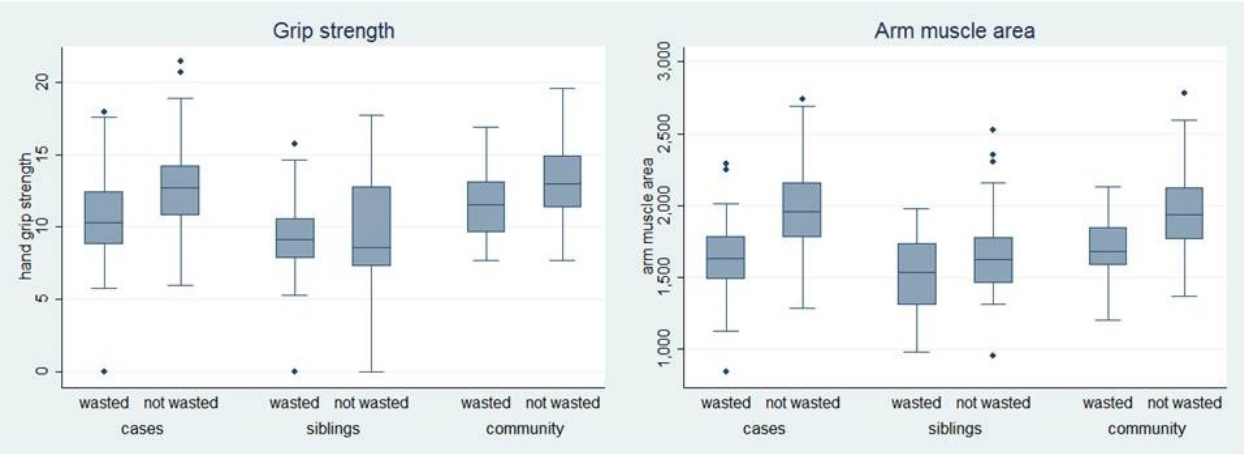


Figure 25: Box plots for hand grip strength and AMA stratified by study group and WAZ category. Wasted defined as WAZ<-1.5

6.4.4.2 Lean Mass

There is a significant positive association between hand grip strength, AMA, time completed of iStep and lean mass (measured either using LMI or R/H). Interestingly, there is also an association between steps per hour and lean mass, indicating that lean mass as assessed by BIA may be a good measure of physical activity (results in **Table 29**). Causality i.e. whether more lean mass results in greater physical function or vice versa, cannot be deduced from this data.

	Hand grip strength (kg) Unadjusted difference (95% CI)	**AMA (cm^2) Unadjusted difference (95% CI)**	**Time completed of iStep (minutes) Log-odds (95% CI)**	**Steps per hour Unadjusted difference (95% CI)**
LMI	0.75 * (0.4, 1.1)	189.2 * (151, 228)	0.31 * (0.1, 0.5)	88.59 *(35,141)
R/H	-0.03 * (-0.0, -0.0)	-3.69 * (-3.9, -3.4)	-0.01 *(-0.0, -0.0)	-0.67* (-1.2, -0.1)
Xc/H	-0.25 * (-0.3, -0.2)	-30.1 * (-34, -25)	-0.04* (-0.1, -0.0)	-7.38 (-14.9, 0.2)

Table 29: Results of regression analysis of functional outcomes against lean mass indicators for the cohort as a whole.

6.4.5 Associations between Physical Function Measures

There is a significant positive correlation (after adjusting for age and sex) between:

- Hand grip strength and physical capacity (p=0.004)
- Hand grip strength and AMA(p<0.001)

There is no significant association between physical activity and any of the other physical function measures, however sample size for physical activity is small. There is also no significant correlation between AMA and physical capacity; however there is an association between physical capacity and calf circumference (log-odds 0.18, 95% CI 0.1 to 0.3, p<0.001).

6.4.6 Predictors of Poor Physical Function in Cases

In order to identify any factors at admission that may predict poor long-term physical function a variety of potential predictors were explored, comparing within the case group. Any predictors that were identified as potentially significant where explored further by comparing the two types of cases to controls (siblings and community controls combined).

Table 30 shows adjusted difference within the case group for hand grip strength, AMA and time completed of iStep test comparing various potentially predictive factors. Results for steps per hour have not been presented as sample size is too small, especially for stratification. Those cases that were severely stunted or severely wasted at admission have weaker handgrip strength, smaller AMA but no difference in physical capacity at 7 years post-discharge. However it is important to remember that those severely stunted and wasted at admission continue to be more stunted and wasted at follow up, which is likely impacting on result as mentioned above.

Those who were admitted with oedema have similar handgrip strength and similar physical capacity to those admitted without oedema, but oedematous admissions have larger AMA than marasmic admissions at follow-up. This is in line with BIVA results presented in previous chapter that show marasmic admissions continuing to have less lean mass at follow up, whereas kwashiorkor survivors now have similar levels of lean mass to controls.

There is no significant difference in hand grip strength, AMA or physical capacity when comparing HIV status, sex and reported birth weight, within the case group. There is no significant difference between those admitted before 24 months and those admitted after for handgrip strength or AMA. Interestingly, those admitted after 24 months completed significantly more minutes of the exercise test, despite having worse growth outcomes.

Potential Predictors	Adjusted difference Hand grip strength (95% CI)	Adjusted difference AMA (95% CI)	Adjusted Log odds Time completed (95% CI)
Stunted at admission (HAZ <-3)	-0.98 * (-1.9, -0.0)	-86.2 (-196, 23.8)	0.24 (-0.3, 0.8)
Wasted at admission (WAZ≤-4)	-1.16 * (-1.9, -0.4)	-148.8 * (-241, -56.5)	-0.43 (-1.0, 0.1)
Oedema at admission	0.55 (-0.5, 1.6)	141.0 * (16.4, 266)	0.06 (-0.7, 0.8)
HIV negative vs positive	-0.03 (-0.9, 0.8)	3.35 (-97.5, 104)	-0.27 (-0.8, 0.3)
Females vs males	0.75 (-0.0, 1.5)	-20.2 (-111.7, 71.3)	-0.16 (-0.7, 0.4)
Normal vs low birth weight	-0.14 (-1.6, 1.3)	-85.3 (-250.6, 79.9)	0.03 (-0.9, 1.0)
Admitted ≤2 yrs vs >2 yrs	0.07 (-0.9, 1.1)	-48.0 (-164, 67.9)	1.07 * (0.3, 1.8)

Table 30: Results of linear regression and ordered logistic regression analysis comparing characteristics at admission within the case groups adjusted for age, sex, HIV status and SES

When compared to controls (siblings and community combined) both oedematous and marasmic admission have lower hand grip and AMA than controls; equally both those severely stunted at admission, those less stunted at admission, those severely wasted at admission and those less wasted at admission have lower hand grip, AMA and time completed of iStep than controls. For age at admission, both younger admissions and older admissions have significantly lower hand grip and AMA than controls, but only those admitted before 24 months completed significantly fewer minutes of the iStep test (log odds -0.49, p=0.04 (adjusting for age and HIV status). There is no significant difference in time completed of the iStep test for those admitted after 24 months (log odds -0.24, p=0.36)

6.5 Summary

This chapter explores 3 main areas of physical function: muscle strength, physical activity and physical capacity. Results show no consistent difference for reported physical disabilities between cases and controls. Cases have significantly smaller AMA than both sibling and community controls, as well as weaker hand grip strength. Cases may have lower physical capacity than community controls as their heart rate recovers significantly more slowly following the exercise test. There is no significant difference in oxygen saturation between cases and controls during exercise. Cases are less physically active than controls (steps per hour) however sample size is too small to be conclusive. Interestingly, after adjusting for case/control status, HIV has no significant impact on hand grip strength, AMA, physical activity or physical capacity. Weight and height do affect hand grip strength and AMA but not physical capacity; results suggest that catching up height does not mitigate the long-term effects of SAM on physical function, however catching up weight does. Lean mass as measured by BIA is an important indicator of physical function as it is significantly associated with all outcomes in this chapter. Greater stunting and greater wasting at admission are associated with worse muscle strength and lower AMA 7 years post-discharge, likely due to the continued deficits in WAZ and HAZ in these patients. Marasmic admissions, rather than those who had kwashiorkor, continue to have lower AMA however this doesn't seem to have affected muscle strength or physical capacity.

7 Chapter 7: Lung function

This chapter introduces lung function and specifically the potential effects that SAM could have on long-term lung function. Continuing on from the previous chapter which explored physical function, lung function is another important functional outcome and could also indicate the health of vital organs in general. This chapter also describes the specific methods related to lung function outcomes, analysis and lung function results. Methods and results from this chapter have been presented at European Respiratory Society (ERS) conferences in 2014 and 2015.

7.1 Chapter-specific Hypotheses

Lung development including alveologenesis, lung size and gas-exchange surface area occurs between gestation and early childhood[32, 163]. An insult to growth and development during any of these time periods could feasibly damage lung function. There is already evidence that intrauterine insults, nutritional and other, can result in poor lung function and increased risk of asthma in childhood and heightened risk of COPD in adulthood[32]. Based on this evidence, I hypothesised that:

5. Compared to controls, SAM survivors have impaired lung function; namely
    a. reduced Forced Expiratory Volume in 1 second (FEV_1)
    b. reduced Forced Vital Capacity (FVC)
    c. and/or reduced FEV_1/FVC ratio z scores.

    Additionally, those who suffered SAM at a younger age (<2 years) have greater lung function impairments.

7.2 Measuring Lung Function

Spirometry testing is the most common method of measuring lung function. Although it does not assess all aspects of lung function, it does assess the volume and flow of air that can be inhaled and exhaled. The main outcomes are:

- Forced expiratory volume in 1 second (FEV_1)
- Forced vital capacity (FVC)
- FEV1/FVC ratio
- Forced expiratory flow (FEF)
- Mean forced expiratory flow during 25-75% FVC (FEF_{25-75}).

The FEV_1 is the volume exhaled during the first second of a forced expiratory manoeuvre; FVC is the total amount of air expelled from the lungs after a maximal inhalation. The FEV_1 is reduced in both obstructive and restrictive lung disease; these two categories of lung disease are differentiated using FEV1/FVC ratio as FVC is only reduced in restrictive disorders. In asthma for example, an obstructive lung disorder, the FEV_1 is usually decreased but the FVC is normal hence the FEV_1/FVC ratio is decreased. Both obstructive and restrictive lung function patterns could be relevant to SAM survivors. FEF is the speed of the air coming out of the lung during the middle portion of a forced expiration; it has recently been suggested that FEF_{25-75} is a more sensitive measure of obstructive small airways disease[245].

Other lung function tests include gas diffusion tests which measure amount of oxygen or carbon dioxide in the blood stream; body plethysmography which measure total lung capacity and residual volume using a plethysmograph booth which measures pressure changes as you breath; and exercise tests which measure lung diffusion capabilities by monitoring blood oxygen saturation during exercise.

7.3 Specific Methods

7.3.1 Anthropometry

Chest circumference: this was measured after the child had removed clothing from the chest, and stood with their hands by their side. The tape was passes around the chest, 2cm below the arm pit but always above the nipple. The tape should be horizontal with the floor and the measurement taken during a normal exhale; this measurement sometimes included protruding shoulder blades depending on the child's chest shape.

Chest width: this was measured at the same level as chest circumference, using body callipers. The child stood with relaxed shoulders and with their hands on their hips while the measurement was taken from the child's back.

Chest depth: this was measured using the same body callipers as with chest width, and the reading was taken at the same level as both chest width and chest circumference. The child stood with their hands by their sides and can be approached with callipers from either side. It was important that the callipers were horizontal with the floor as this could often be difficult with certain shoulder blade shapes.

7.3.2 Spirometry

The spirometry testing was done using an NDD Easy On-PC spirometer (NDD Medical, Switzerland). "FVC (exhale only)" test was used. This device was chosen as it connects directly into a PC or laptop, making it highly portable and it has an ultrasonic flow head which allows for easy calibration in the form of zero flow verification. It also has the added benefit of displaying spirometry-related incentives (i.e. children's computer games) to encourage optimal exhalations, as spirometry is an effort-dependant test. Effort, as well as skill, is also required from the operator of the test to encourage maximal effort from the children; I undertook 2 weeks of training at Great Ormond Street children's hospital in London by a team from the Institute for Child Health in order to learn how best to conduct spirometry tests with children. I then passed this training on to select members of the Malawian study team. We also shadowed the Blantyre Health Study team who were undertaking spirometry in Malawian adults in order for us to learn appropriate Chichewa translations for common spirometry instructions.

7.3.2.1 Contraindications

A questionnaire regarding contraindications and confounding factors was administered to the parent or guardian of the child prior to conducting spirometry, including questions regarding abdominal aneurysm, recent pneumothorax, asthma, eczema, environmental smoke exposure and history of respiratory illness. These questions were developed based of questions from "American Thoracic Society's ATS-DLD-78C respiratory questionnaire" and UCL's "SLIC" study

questionnaire[246, 247]. Spirometry was not attempted if the child had any one of the following contraindications:

- abdominal aneurysm
- recent heart attack
- current respiratory infection
- recent pneumothorax
- haemoptysis
- any recent surgery

7.3.2.2 Spirogram Acceptability and Grading

During the spirometry test, acceptability of traces was assessed as to know when to stop testing. Ideally the testing finished when there were 3 repeatable readings, however this was not always possible. The operator never ended the test on the best attempt in case the child could improve further. In most cases, at least 6 spirometry traces were recorded for each child; in some cases as many as 25 spirometry attempts were done by one child.

The criteria for acceptability of individual spirometry traces are listed below. **Figure 26** gives an example of an acceptable and an unacceptable trace. Although the NDD software automatically rejects unacceptable traces and selects the child's best result for export, this process is not perfect and needs to be checked. Inappropriate traces were discounted and acceptable ones included by the operator. I also checked all spirometry data each week and Jane Kirkby (senior respiratory physiologist, UCL Respiratory Section IIIP), who was blinded to the study groups, checked at least 10% of results each month, amending acceptable and unacceptable inclusions. The final spirometry outcomes are based on the best attempt of all the "acceptable" traces.

Criteria for acceptable spirometry traces:

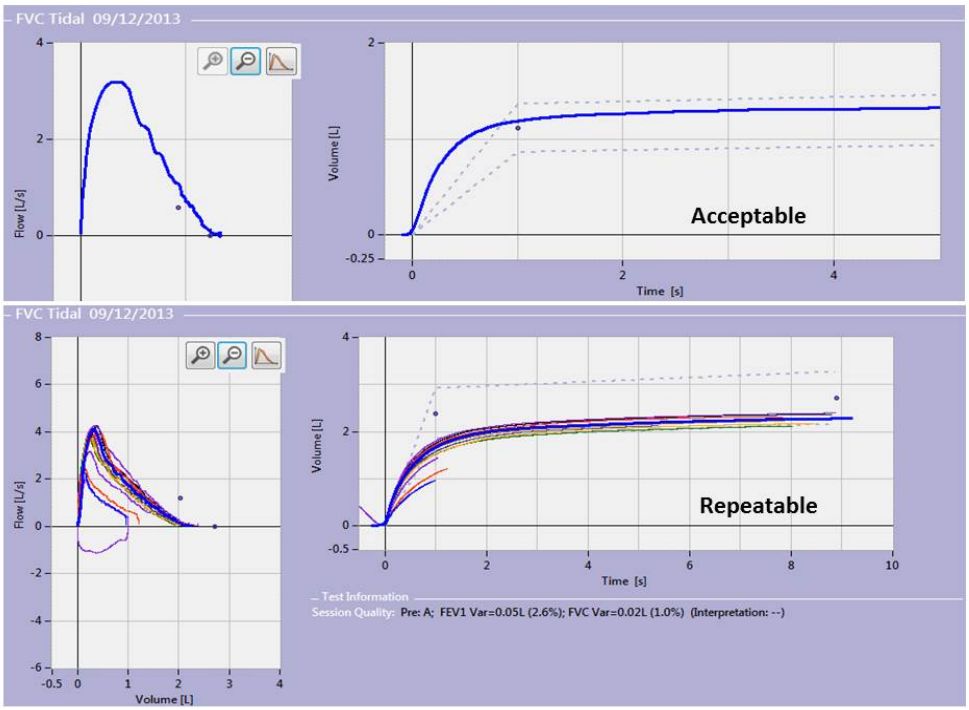
- Free from artefact i.e.
    - Coughing
    - Glottis closure
    - Early termination or cut-off
    - Leak
    - Obstruction of the mouth piece (tongue or biting)
- Effort must be maximal throughout
- Exhale start should be strong
- Back extrapolated value should be <5% of FCV
- Exhale should last at least 3 seconds or plateau in the volume-time curve

Following this quality control process, quality grades were applied to the data for each child based on repeatability of accepted traces (see **Table 31** for grades and repeatability criteria). The criteria for repeatability of the spirometry curves was based on "ATS/ERS task force: standardisation of lung function testing" by Miller et al. adapted slightly for children to accept a 10% difference between spirograms in accordance with guidelines used by Kirkby et al [248, 249]. This grading system allows one to distinguish between a child who has a poor spirometry reading but only one acceptable trace, and another who has a poor spirometry reading with multiple acceptable, similar traces to back it up, proposing that better grades are more likely to be a true reflection of lung function than lower grades.

Grade	FEV_1 or FVC
A	3 acceptable trials within 5% or 100mL
B	2 acceptable trials within 5% or 100mL
C	2 acceptable trials within 10% or 150mL
D	One acceptable trial
F	Fail: No acceptable trials

Table 31: Quality Control Grading for Spirometry

a)



b)

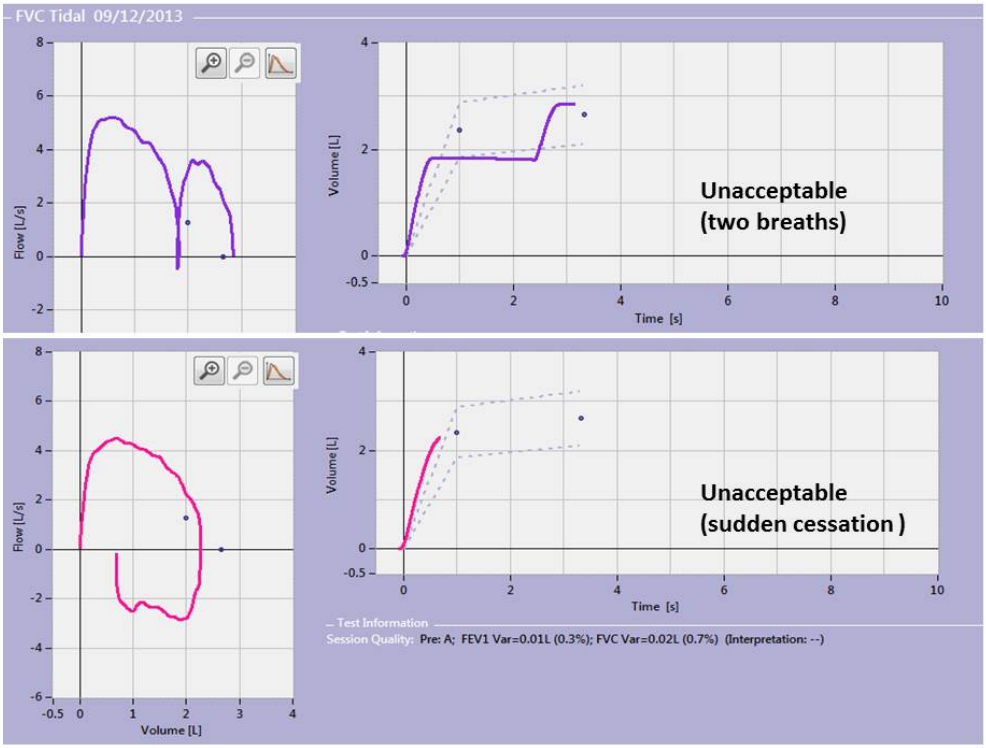


Figure 26: Examples of a) acceptable and repeatable spirometry traces b) unacceptable traces

7.3.3 Sample Size Calculations

Sample size for this study was difficult to calculate as reference ranges for Malawian children do not yet exist. Based on a common clinical difference in lung function of 0.5 z scores (standard deviation 1.0), I calculated that sample size needed to be 85 in each group to detect this difference. A follow-up study of lung function in Nepal based their sample size calculation on US data from children in the National Health and Nutrition Examination Survey III[250, 251] and suggested that only a 0.2 z score difference should be expected between groups. To detect this difference and based on our sample size of 199 cases and 142 siblings, our sample is only powered to 44% and analysis of community controls (n=121) is powered to 35%.

Image 15: Study children undertaking spirometry testing on the NDD Easy-On PC spirometer during the data collection in Blantyre

7.4 Analysis

Primary analysis compared sibling and community controls to cases using regression analysis for:

- Chest anthropometry
- Main spirometry outcomes: FEV_1, FVC and FEV/FVC ratio z scores

As these are all continuous outcomes, simple and multivariable linear regression analysis was used to compare cases and both types of control. Potential confounding factors were adjusted for in multivariable linear regression; namely HIV status, sitting height, puberty status and SES, based on *a priori* assumptions. Age and sex were also included in anthropometry outcomes as they have not been converted to z scores. A description of how potential confounders were selected for inclusion is provided below.

Secondary analysis considered impact of various other factors on spirometry outcomes including HIV status, wealth, sex and effect of being in a "SAM" household. I also explored if any factors at admission were predictive of poor lung function 7 years post-discharge, namely severity of stunting and wasting at admission, HV status, age at admission and presence of oedema, as well as reported birth weight, for case children only. Besides linear regression, Students t test and Chi squared testing were also used in some analyses in this Chapter to assess association when only two groups are involved and/or if both variables are categorical.

7.4.1 Data Cleaning

As with growth data, I check for erroneous values among the anthropometric measures by plotting scatter graphs of each variable against age, height and weight. Outliers were identified and paper files check for notes, corrections or explanations. Impossible readings without explanation were removed: 2 chest circumference readings. No chest width or chest depth data were removed. I removed spirometry data for 1 child due to implausibility; this result was at the extreme high end of z scores for age and height and was not repeatable; it was most likely the result of the operator or another older child demonstrating an attempt.

7.4.2 Calculations and Statistics

The analysis of spirometry data focused on the most widely used spirometry outcomes: FEV_1, FVC, and FEV_1/FVC ratio. For all children with grade A-D spirometry results, the best acceptable spirometry reading was used to calculate z scores based on child's age, height, sex and ethnicity using Quanjer et al., (2012) Global Lung Function Initiative (GLI) reference data [252]. The GLI reference was derived from healthy individuals between ages of 3 and 95, including Caucasian, African-American, North East Asian and South East Asian ethnicities. Z scores were computed for Malawian children using the African-American only reference data (called GLI-Black). There

is currently no specific spirometry reference for Malawi or Southern Africa; one small study considered spirometry outcomes in Malawian children and showed them to be lower than both Caucasian and conventional Black reference data, however because I am comparing cases to controls, the appropriateness of the reference equation to Malawian children in general is less important [253]. The applicability of the GLI-Black equation for these Malawian children was investigated in a separate study, abstract and poster of which can be seen in **Appendix 22**.

The following calculations were applied to the anthropometry data:

- **Sitting height percentage** = (sitting height / height)*100
- **Relative chest circumference** = chest circumference / height

7.4.3 Adjusting for Potential Confounders

Potential confounding factors were adjusted for in multivariable linear regression; namely HIV status, age, sex, puberty and socio-economic status (SES). These variables are highly likely to be confounders as they are closely linked to SAM and likely linked to lung function; hence they were controlled for regardless of statistical significance. For exploring the association between SAM and spirometry outcomes, further potential confounders including previous respiratory infection, sitting height percentage, reported asthma, maternal education level and household smoke were considered. There is evidence that up to 35% of those with COPD in low and middle income countries developed the disease after exposure to biomass fuel combustion, hence this is an important consideration[254]. Previous respiratory infections are also important to consider as evidence shows they are closely linked to COPD in later life, however the direction of causality is not yet clear[32, 73]. One confounder that I was not able to adjust for is birth weight, as this is not known for the majority of study participants, though is known to have significant effects on later lung function[73]. Lung function is also closely linked to sitting height because, although z scores account for overall height, they do not account for differences in torso height specifically, which is likely to be more closely related to lung volume. A conceptual framework of all these potential confounders is depicted in Figure 4d, Chapter 2. **Table 32** shows consideration of all these potential confounders in relation to FEV_1, FVC and FEV_1/FVC z scores; previous respiratory infection, maternal education level and household smoke exposure are not associated with spirometry outcomes, hence were not included in multivariable linear regression as potential confounders. Reported Asthma is only significantly associated with FEV_1/FVC ratio. It is worth mentioning that only 1.7% of our sample cook with a clean fuel and only 1.5% do not cook on an open fire, hence there is likely to be little variation in cooking smoke exposure across the sample. Sitting height percentage is positively associated with both FEV_1Z and FVCZ therefore it is

included in multivariable regression analysis when comparing lung function outcomes between groups in this chapter.

	FEV_1Z	FVCZ	FEV_1/FVC Z
	Adjusted difference (95% CI)	**Adjusted difference (95% CI)**	**Adjusted difference (95% CI)**
HIV positive	-0.51 * (-0.8, -0.2)	-0.43 * (-0.7, -0.1)	-0.22 (-0.5, 0.1)
Age (years)	-0.12 * (-0.2, -0.1)	-0.09 * (-0.1, -0.0)	-0.08 * (-0.1, -0.0)
Started puberty	0.30 (-0.2, 0.8)	0.30 (-0.2, 0.8)	0.11 (-0.3, 0.5)
SES	0.10 * (0.0, 0.2)	0.08 * (0.0, 0.1)	0.02 (-0.0, 0.1)
History of pneumonia	0.22 (-0.2, 0.7)	0.16 (-0.3, 0.6)	0.08 (-0.3, 0.5)
Reported asthma	-0.13 (-0.5, 0.3)	0.11 (-0.3, 0.5)	-0.38 * (-0.7, -0.0)
Maternal education (2^{ndary} vs none)	-0.02 (-0.4, 0.3)	0.02 (-0.3, 0.40	-0.16 (-0.5, 0.2)
Cooking with solid fuel in the home	0.30 (-0.0, 0.6)	0.27 (-0.1, 0.6)	-0.02 (-0.3, 0.3)
Sitting height percentage	0.15 * (0.1, 0.2)	0.12 * (0.1, 0.2)	0.06 * (0.0, 0.1)

Table 32: Results presented for linear regression analysis of each potential confounder for FEV_1, FVC and FEV_1/FVC z scores. Results are all adjusted for study group (i.e. case/control status). * indicates $p<0.05$

7.5 Results

7.5.1 Missing Data

Spirometry was attempted by 532/721 (74%) children in the study (see flow diagram in **Figure 27**). 189 children did not attempt spirometry for the following reasons:

- Child was less than 6 years old (only applies to sibling controls) (n=19)
- Child was too unwell (e.g. malaria or a current respiratory infections) (n=36)
- Child didn't attend the hospital appointment because they didn't want to or they lived too far away (n=95)
- Child has a physical and/or cognitive disability (n=39)

For a full table of number of results for each outcome variable see Table 4 in Chapter 3: Cohort Profile.

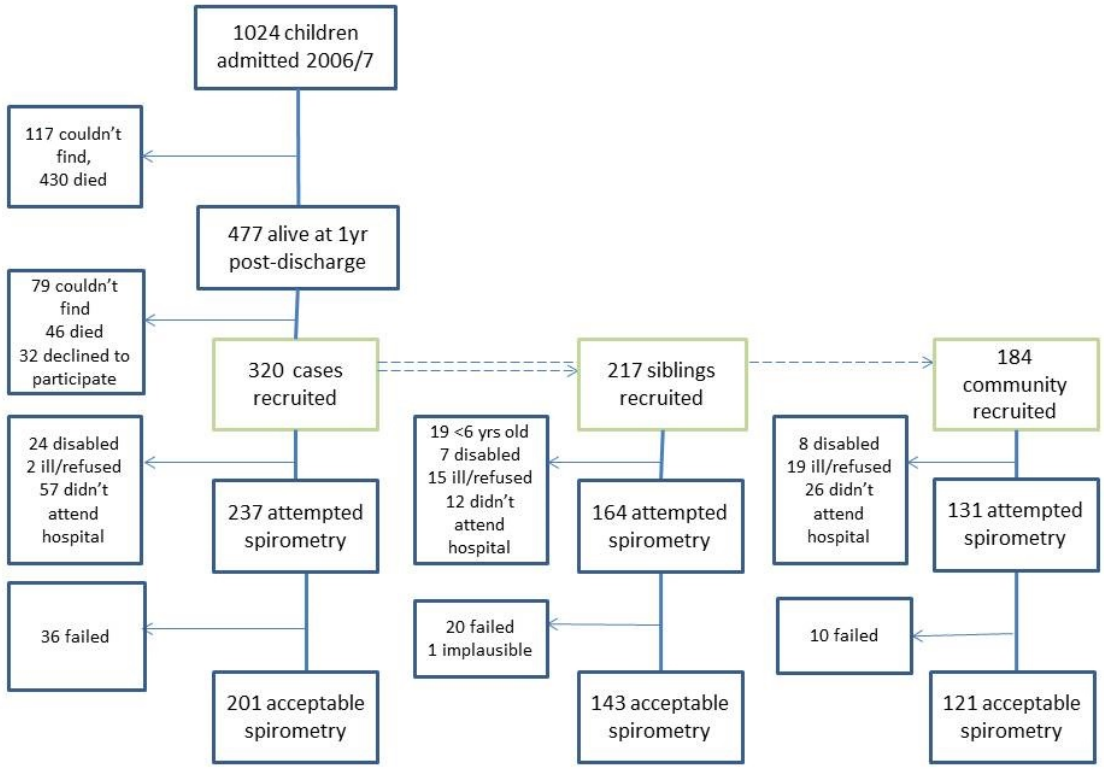


Figure 27: Flow diagram showing recruitment of cases, siblings and community controls for spirometry outcomes

In order to explore any "response bias" caused by this missing data, **Table 33** shows results of a Student's t-test comparing age and anthropometry data for those who did vs those who didn't attempt spirometry testing. There is no significant difference in WAZ, HAZ, BAZ or sitting height percentage between the 2 groups. There is also no difference in HIV status or gender balance between the two groups. There is however a significantly higher proportion of children with disabilities in the "didn't attempt" group (7% vs 1%) (Chi squared 20.4, 1 d.f, $p<0.001$), and the "didn't attempt" group are slightly but significantly younger, which is as expected as age was an exclusion criterion ($p=0.02$).

There is also a significant relationship between those who attempted spirometry and study group; the odds of attempting spirometry are 1.8 ($p<0.001$) for siblings compared to cases, and 1.5 ($p=0.05$) for community controls compared to cases. This is likely due to the higher prevalence of disability in the case group; of the 45 with disabilities who couldn't attempt spirometry 43 were cases, 2 were siblings and 0 were community controls.

	Didn't do spirometry Mean (SD)	Did spirometry Mean (SD)	Mean difference (95% CI)	*p* value
Age (years)	9.6 (2.3)	10.0 (2.1)	-0.4 (-0.7, -0.1)	0.02 *
WAZ	-1.4 (1.0)	-1.4 (1.0)	-0.1 (-0.4, 0.1)	0.3
HAZ	-1.7 (1.3)	-1.6 (1.2)	-0.1 (-0.3, 0.2)	0.5
BAZ	-0.7 (1.0)	-0.8 (0.9)	0.1 (-0.1, 0.3)	0.4
Sitting height percentage (%)	52.1 (1.8)	52.0 (1.5)	0.2 (-0.1, 0.5)	0.2

Table 33: Mean (SD) and results of Student t-test for those who did attempt spirometry vs those who did not (continuous variables only). * indicates $p<0.05$

7.5.2 Data Quality

Of 532 children with spirometry results, 364 had repeatable data (Grade A-C) for both FEV_1 and FVC (see **Table 34** for breakdown of data quality by study group). The overall fail rate was 12% (64/532) (Grade F). Grade F data was excluded from analysis. 14% (72/532) achieved Grade D (1 acceptable trace) for FEV_1 and 12% (62/532) for FVC. There is no statistically significant relationship between being a case or control and spirometry quality grade achieved (A-C, D or F) (chi-square with 4 degree of freedom FEV_1= 5.33, p= 0.3; FVC = 8.52, p=0.07).

	Ex-SAM Case n=237	**Sibling Control** n=164	**Community Control** n=131
FEV_1 & FVC repeatable (A-C)	146 (62%)	116 (71%)	102 (78%)
Symptomatic	13 (5%)	4 (2%)	4 (3%)
FEV_1 only	15 (6%)	5 (3%)	6 (5%)
FVC only	2 (1%)	1 (0.6%)	0
Grade D	25 (11%)	16 (10%)	9 (7%)
Fail	34 (14%)	20 (12%)	10 (8%)
Fail & symptomatic	2 (1%)	0	0
Exclude:Implausable	0	1 (0.6%)	0

Table 34: Break-down of quality control information by study groups

Children with reported coughs/colds ('symptomatic'), self-reported asthma, cooking with solid fuel in the home or history of pneumonia and those with grade D data were reviewed to ascertain if they could bias our results. **Figure 28** shows that both symptomatic and Grade D children have FEV_1 and FVC z scores evenly spread throughtout the range of results i.e. they are not at the extremes of the z scores. **Table 35** summaries the mean (SD) z scores for quality data (grade A-C) and grade D data, sympotmatic data and children with history of respiratry illness; linear regression analysis revealed no statistical group differences in FEV_1, FVC nor FEV_1/FVC z scores between children with grade D data/symptomatic/history of pnuemonia/self-reported asthma/cooking smoke in home, and quality data. There is however a significant difference in FEV1 and FVC z scores between those with a history of TB and quality data (FEV_1Z Coeff -0.91, p<0.001; FVCZ Coeff -1.1, p<0.001). Inclusion of grade D and symptomatic data in the final analysis therefore does not bias the results and has been included; those with a history of TB have been excluded from analysis. Inclusion of grade D and symtomatic data is inline with some previous studies[255] .

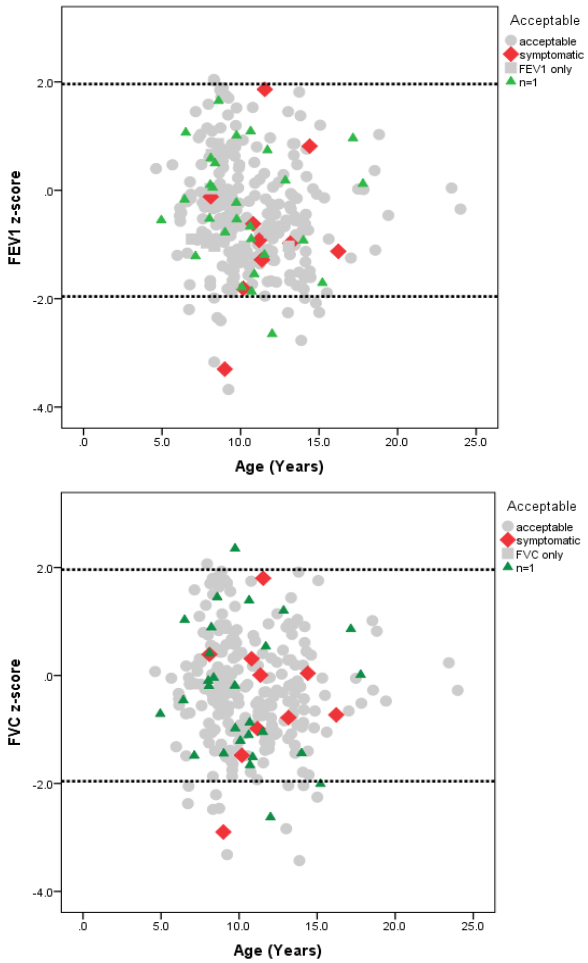


Figure 28: The distribution of FEV_1 (left) and FVC (right) z scores for all quality results and those which came from Grade D children (green triangles) and symptomatic children (cold/flu symptoms) (red diamonds).

	Grade A-C (n=364)	**Grade D** (n=50)	**Symptomatic** (cough/cold) (n=23)	**History of pneumonia** (n=25)	**Self-reported Asthma** (n=28)	**History of TB** (n=11)	**Cooking with solid fuel in the home** (n=51)
FEV_1 z-score	-0.4 (1.0)	-0.4 (1.1)	-0.7 (1.3)	-0.2 (1.3)	-0.5 (1.0)	-1.3 (1.7)	-0.2 (0.9)
FVC z-score	-0.3 (1.0)	-0.4 (1.2)	-0.2 (1.2)	-0.2 (1.2)	-0.2 (0.8)	-1.3 (1.7)	-0.1 (0.9)
FEV_1/FVC z-score	-0.2 (0.9)	0.1 (0.9)	-0.9 (1.0)	-0.2 (1.0)	-0.6 (1.3)	-0.6 (1.3)	-0.3 (0.8)

Table 35: Mean (SD) FEV_1, FVC and FEV_1/FVC z scores for acceptable quality data (Grade A-C) versus grade D data, symptomatic children, children with a history of pneumonia, history of TB, cooking smoke in the home and those with self-reported asthma. The results indicate that all these groups have similar mean z scores to acceptable data, except those children who have a history of TB.

7.5.3 Anthropometry

As stated in Chapter 4: Growth, case children are significantly more stunted and have larger sitting height percentage than both sets of controls. **Table 36** shows results of simple and multivariable linear regression analysis comparing chest anthropometry of cases and control groups; results indicate no significant adjusted difference in chest size or torso height, apart from chest width which is significantly larger for controls than cases. This information indicates that although cases are shorter for their age than controls, their sitting height and chest size is largely preserved when compared to sibling and community controls. Histograms for each variable can be found in **Appendix 23**.

Anthropometry	**Cases** (n=302)	**Sibling** (n= 202)			**Community** (n=158)		
	Mean (SD)	**Mean** (SD)	**Unadjusted difference** (95% CI) *P value*	**Adjusted difference** (95% CI) *P value*	**Mean** (SD)	**Unadjusted difference** (95% CI) *P value*	**Adjusted difference** (95% CI) *P value*
Chest width (cm)	19.1 (1.5)	19.9 (2.6)	0.81 * (0.5, 1.2) *<0.001*	0.39 * (0.1, 0.7) *0.005*	19.3 (1.7)	0.28 (-0.1, 0.7) *0.16*	0.34 * (0.0, 0.6) *0.02*
Chest depth (cm)	14.4 (1.4)	14.9 (2.0)	0.49 * (0.2, 0.8) *0.001*	0.20 (-0.0, 0.4) *0.11*	14.5 (1.5)	0.09 (-0.2, 0.4) *0.55*	0.17 (-0.1, 0.4) *0.21*
Chest circumference (cm)	60.8 (4.8)	62.8 (7.8)	1.99 * (0.9, 3.1) *<0.001*	0.68 (-0.1, 1.5) *0.09*	61.3 (5.4)	0.55 (-0.6, 1.7) *0.35*	0.82 (-0.0, 1.7) *0.06*
Sitting height (cm)	65.2 (4.4)	67.4 (7.6)	2.2 * (1.2,3.2) *<0.001*	0.6 (-0.1,1.3) *0.11*	65.9 (4.5)	0.8 (-0.3,1.9) *0.18*	0.7 (-0.1,1.5) *0.07*
Chest circumference/ height	0.49 (0.0)	0.48 (0.0)	-0.00 (-0.0, 0.0) *0.13*	-0.00 (-0.0, 0.0) *0.56*	0.48 (0.0)	-0.01 * (-0.0, -0.0) *0.04*	-0.00 (-0.0, 0.0) *0.13*

Table 36: Results of simple and multivariable linear regression analysis for chest anthropometry across the 3 study groups. Adjusted difference includes age, sex, HIV status, SES and puberty. * indicates $p<0.05$.

7.5.4 Spirometry

7.5.4.1 Checking lung function z scores

As raw spirometry data was converted to z scores using African-American reference data, I firstly checked that conversion of raw values into z scores was adequately accounting for differences in age and height. **Figure 29 and Figure 30** show scatter plots for both raw FEV_1 and FVC as well as z scores for control children only; Figure 28 shows spirometry values plotted against age, figure 29 shows spirometry values plotted against height. As you can see from these figures, both age and height are associated with FEV_1 and FVC as expected, and the z score conversion adequately removes any association.

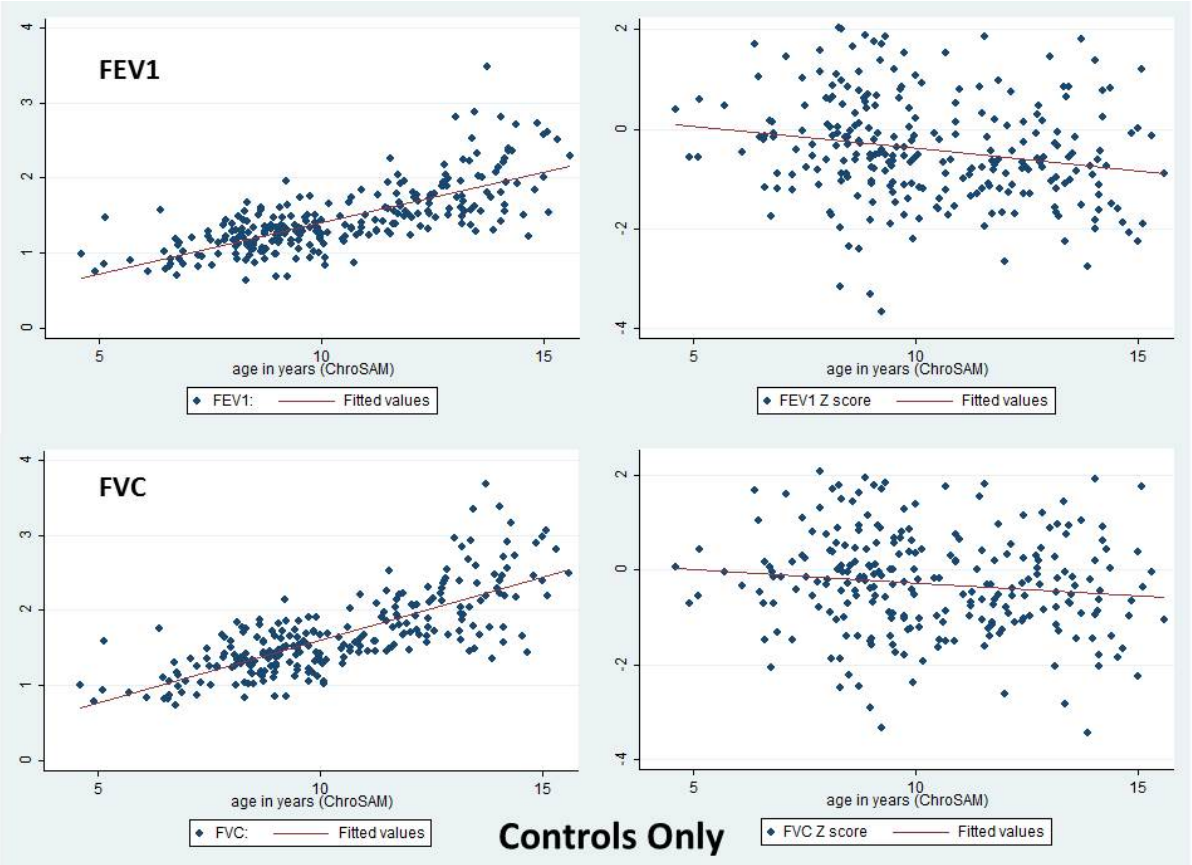


Figure 29: Scatter plots showing raw FEV1 and FVC values against age on the left side, with FEV1 and FVC z scores against age on the right side. This is for control data only to indicate adequate accounting for age by the z score conversions.

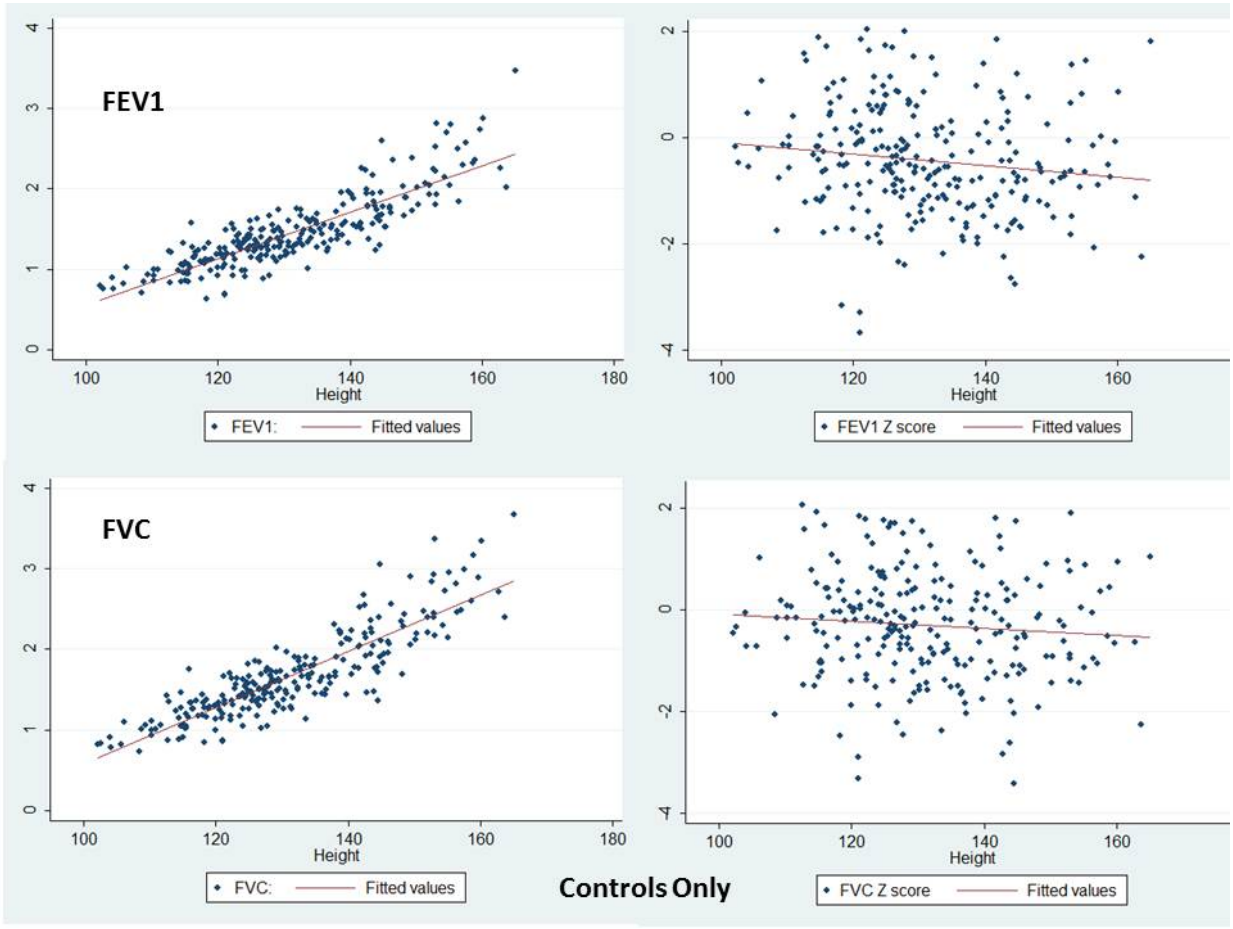


Figure 30: Scatter plots showing raw FEV1 and FVC values against height on the left side, with FEV1 and FVC z scores against height on the right side. This is for control data only to indicate adequate accounting for height by the z score conversions.

7.5.4.2 Lung function of the whole cohort

The sample as a whole is normally distributed for FEV_1Z and FVCZ but has a slight off-set compared to the reference data for a healthy population (expected mean (SD) 0 (1)). Although the standard deviation for our sample as a whole is 1.1 for FEV_1Z and 1.0 for FVCZ, which is a credit to the data quality, the means are slightly below reference mean of 0 (FEV_1Z -0.44; FVCZ -0.29). Histograms for spirometry outcomes can be seen in **Appendix 23**.

When considering the sample as a whole, FEV_1Z and FVCZ are positively and significantly associated with HAZ, WAZ and sitting height percentage, suggesting that underweight and stunted children have worse spirometry outcomes, particularly those with a relatively smaller sitting height.

7.5.4.3 Lung Function and HIV status

This analysis looks at the HIV status for the sample as a whole. There is a significant difference in FEV_1 and FVC z scores between HIV positive and negative children (FEV_1Z regression coef: -0.5, p= 0.001 and FVCZ regression coef: -0.4, p= 0.009). The boxplot in **Figure 31** shows the significant reduction in mean FEV_1 z score for HIV positive children compared to the HIV negative group. HIV does not significantly affect FEV_1/FVC ratio z score (p=0.216) because FEV_1 and FVC are reduced in equal proportions. This indicates that HIV results in a more restrictive lung defect pattern.

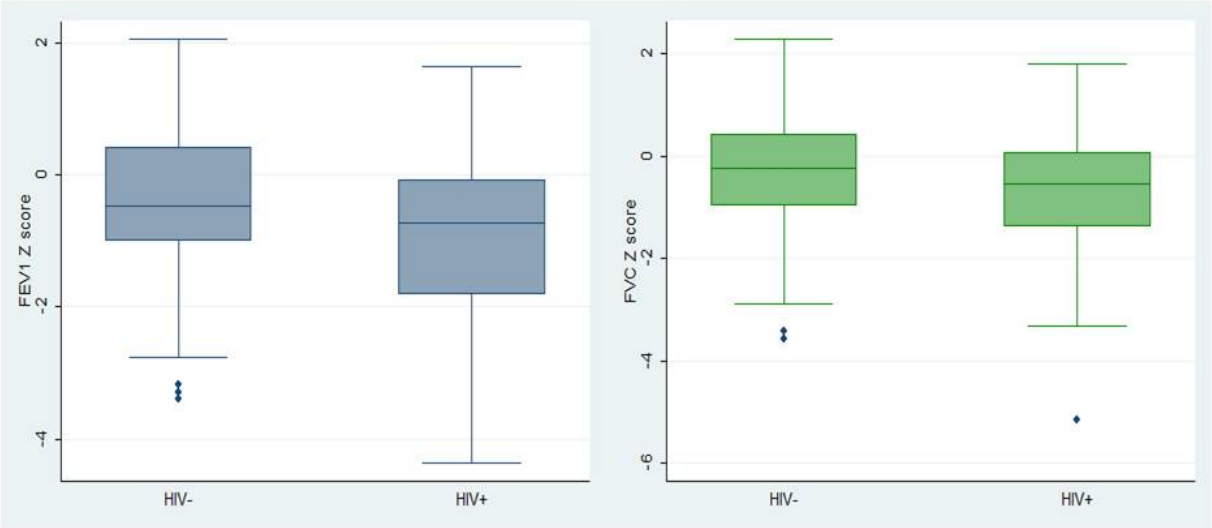


Figure 31: Boxplots of FEV_1 Z (left) and FVCZ (right) for HIV negative and HIV positive groups.

7.5.4.4 Lung Function and Wealth quintile

This analysis looks at the wealth for the sample as a whole. Those in the bottom three wealth quintiles have significantly worse FEV_1 and FVC z scores than those in the top two quintiles (mean difference FEV_1Z -0.30, $p<0.01$; FVCZ -0.26, $p=0.012$). FEV_1/FVC z score is unaffected by wealth quintile. This suggests that poorer children have a slightly more restrictive lung function pattern than wealthier children, although clinically the difference is small. Wealth itself cannot directly affect lung function but must be an indicator of other environmental factors. The bar graph in **Figure 32** shows FEV_1 z score for each of the wealth quintiles (1 is poorest, 5 is richest), highlighting that although there is a statistically significant difference between the wealth quintiles, the difference in z scores is small.

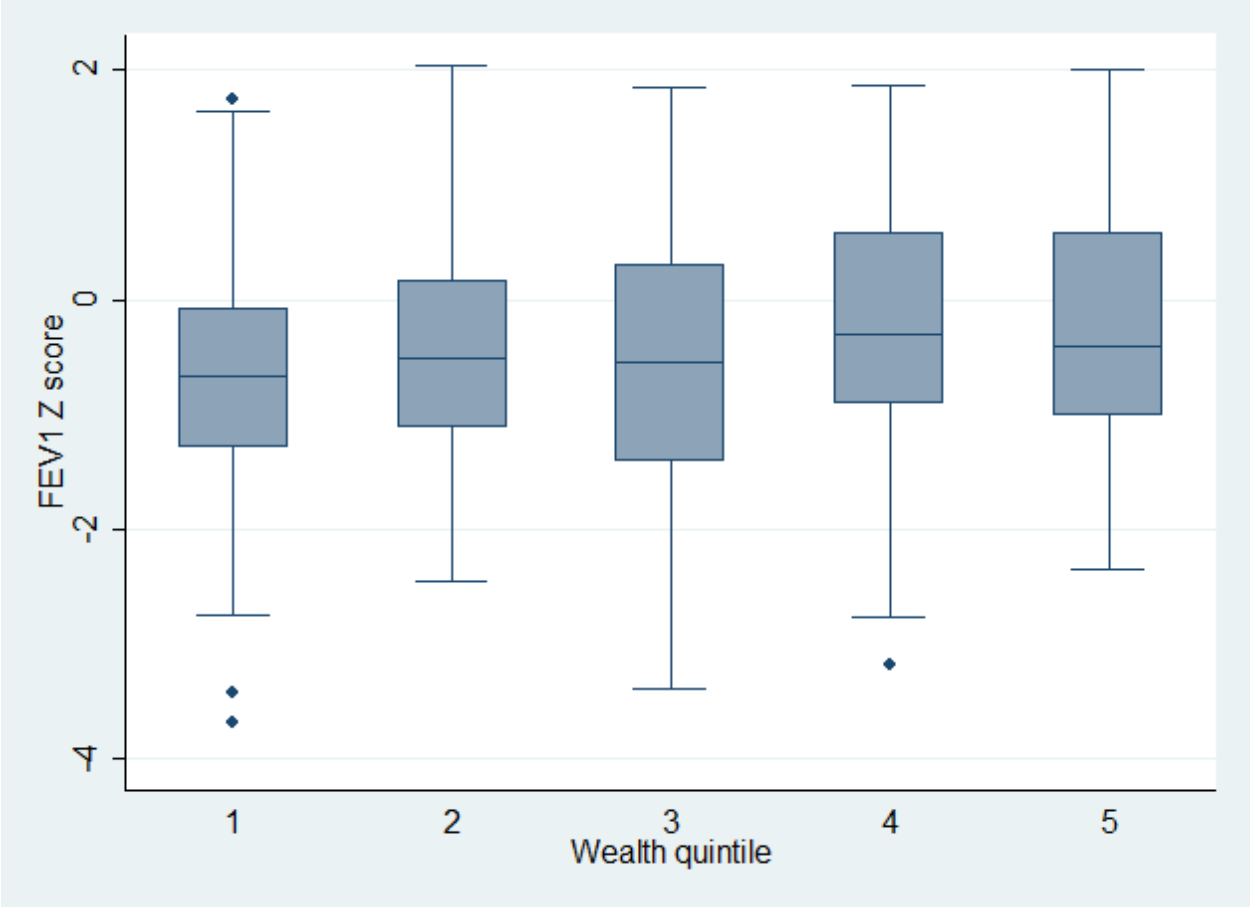


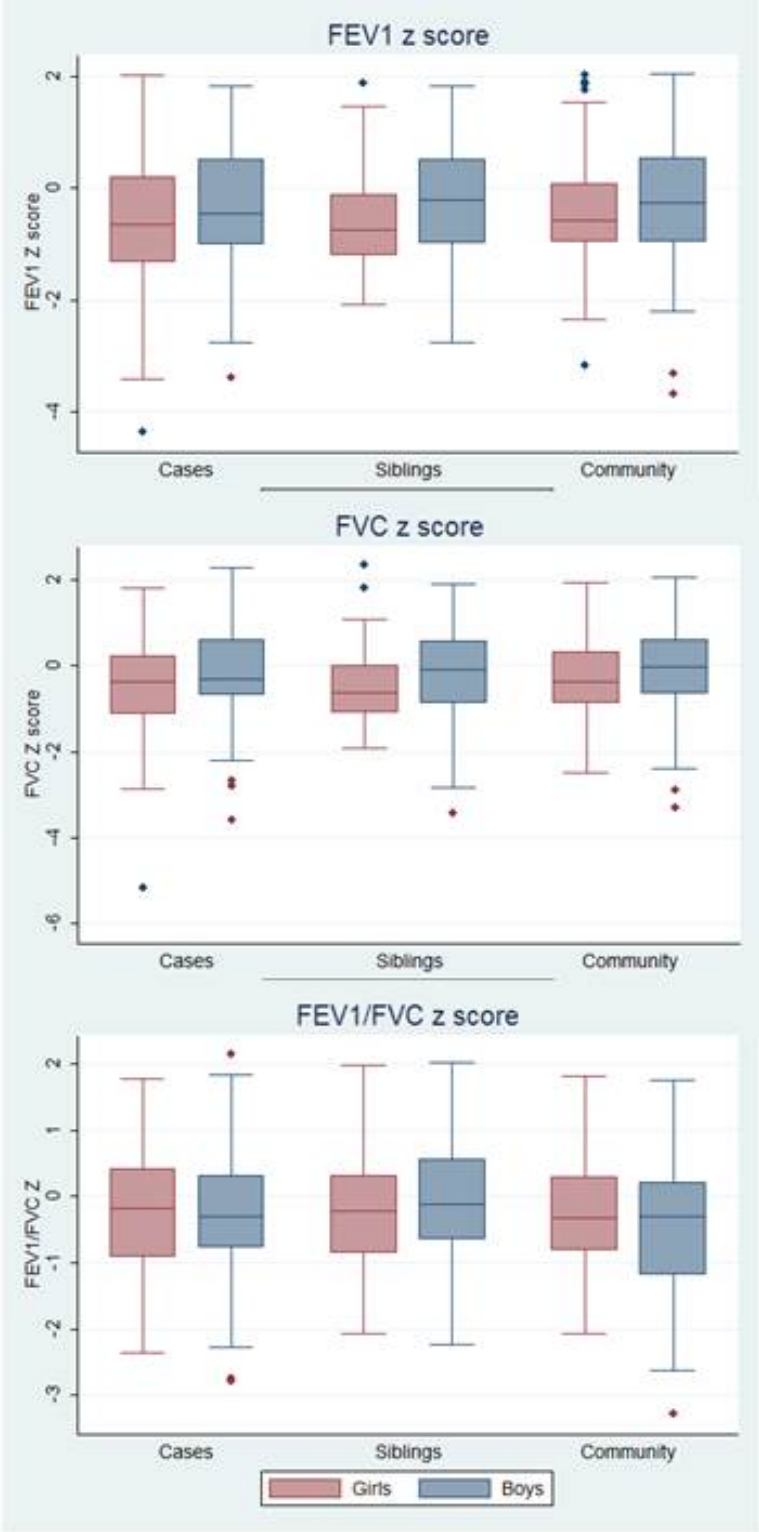
Figure 32: Boxplot showing FEV_1Z for each wealth quintile for the whole sample. Wealth score is an asset-based score derived from Demographic Health Survey questions; score 1 is the poorest and score 5 is the richest within the sample.

7.5.4.5 Lung Function and Sex

This analysis looks at the sex for the sample as a whole. There is no significant difference in proportion of each sex between the case and control groups, however there is a significant difference in FEV_1 and FVC z score between the two sexes, across the whole sample (see **Figure 33**). Females have significantly worse FEV_1 and FVC z scores than males (mean difference FEV_1Z -0.28 p=0.004 and FVCZ -0.25 p= 0.015), but no difference in FEV_1/FVC z score between the sexes. This holds true when using linear regression to adjust for HIV status, SES, sitting height ratio and puberty. There is no significant difference in age, wealth or HIV status between the sexes. Females therefore appear to have a more restrictive lung function pattern than males (reduced FEV_1 and FVC and no difference in FEV1/FVC). Although we have already seen that living with a tobacco smoker and cooking with solid fuel in the home do not significantly affect spirometry outcomes in this study, differential exposure to cooking smoke between the sexes could still be a reasonable explanation for the difference in spirometric score.

Using sex stratified analysis, there is no significant difference in FEV_1 or FVC z scores for males exposed and unexposed to household cooking smoke. However there is a marginal significant difference for females exposed to household smoke having worse FEV_1Z than unexposed girls (coef, 0.50, p=0.058). FVC and FEV_1/FVC z scores are not significantly different in girls exposed to household smoke.

Other explanations for this sex difference that I have explored include delayed puberty in Malawian girls resulting in poor adherence to the African-American reference data differentially for girls, and differences in body composition between sexes affecting lung function. If delayed puberty was affecting the fit to the reference data for girls there would be no sex difference in the younger group (<9 years), however the sex difference remains (FEV_1Z unadjusted difference 0.44, p<0.01; FVCZ unadjusted difference 0.37, p=0.02), so this is an unlikely explanation. Regarding body composition, previous studies have found greater lean mass to be associated with better spirometry outcomes, and greater fat mass with poorer spirometry [256-258]. The females in this cohort have significantly less lean mass and significantly more fat mass than the males; LMI is positively associated with FEV_1Z and FVCZ (FEV_1Z coef. adjusted for case/control status 0.30, p<0.01; FVCZ coef adjusted for case/control status 0.31, p<0.01) whereas fat mass is negatively associated with FEV_1/FVC z ratio (FEV_1/FVCZ difference adjusted for case/control status -0.07, p=0.03). This could therefore be another explanation for sex differences in spirometric results.



n=

	females	males
Cases	98	103
Siblings	76	66
community	51	70

Figure 33: Box plots showing mean FEV_1, FVC and FEV_1/FVC z scores for both sexes and each study group. Females (in pink) have consistently lower FEV_1Z and FVCZ than males across all 3 study groups. FEV1/FVC ratio is unaffected by sex

7.5.4.6 Lung function at Household level

When comparing households (i.e. cases and siblings VS community controls), there is no significant difference in FEV_1Z or FVCZ, with either unadjusted or adjusted regression analysis:

FEV_1Z adjusted difference between case/sibling households and community households is 0.08 (95% CI -0.1 to 0.3) p=0.47. FVCZ is 0.15 (-0.1, to 0.4) p=0.19. FEV_1/FVCZ is -0.20 (-0.4 to 0.0) p=0.06 (adjusted for HIV status, SES and puberty status). Community control households actually have a lower FEV_1/FVCZ ratio than cases and sibling control households with marginal significance.

7.5.4.7 Lung function and SAM; Cases vs Controls

I hypothesised that case children would have poorer FEV_1 and FVC than sibling and community control children due to disrupted lung development during their SAM episode. **Figure 34** shows bee swarm plots for four spirometry z score outcomes for each of the study groups. The plots show an apparent similarity in z scores between the cases and control groups. Simple and multivariable regression analysis for spirometry outcomes confirms that there is no significant difference in FEV_1, FVC and FEV_1/FVC z scores between case and control groups. Results are presented in **Table 37**. There is no significant difference between the groups both when including all the data, and when exclude Grade D data, and when adjusting for HIV status, sitting height ratio, SES and puberty as potential confounders. This also holds true when comparing known HIV negatives only across the groups, as well as stratified analysis by sex (see Tables in **Appendix 24 and 25**).

Also in **Table 37** are means and unadjusted/adjusted differences for raw values of FEV_1, FVC and FEV_1/FVC. The main analysis for this outcome was always intended to be with z scores, however due to the large height difference between cases and controls, it is important to consider these raw values. The important difference between these adjusted values and the z scores is that the volumes are adjusted for sitting height only, rather than over-all height which could be falsely inflating z scores due to stunting in the legs. Sibling controls do have significantly larger FEV_1 even after adjustment, and interestingly, if sitting height is replaced by height in the regression model, the significant difference between cases and siblings for FEV_1 is lost. However there is no significant difference in raw volumes for FVC, nor for any of the outcomes between cases and community controls. Sitting height ratio varies with age, hence the difference in age between cases and siblings could be affecting these results. This suggests that cases have preserved spirometry volumes compared to controls even when only adjusting for sitting height rather than overall height.

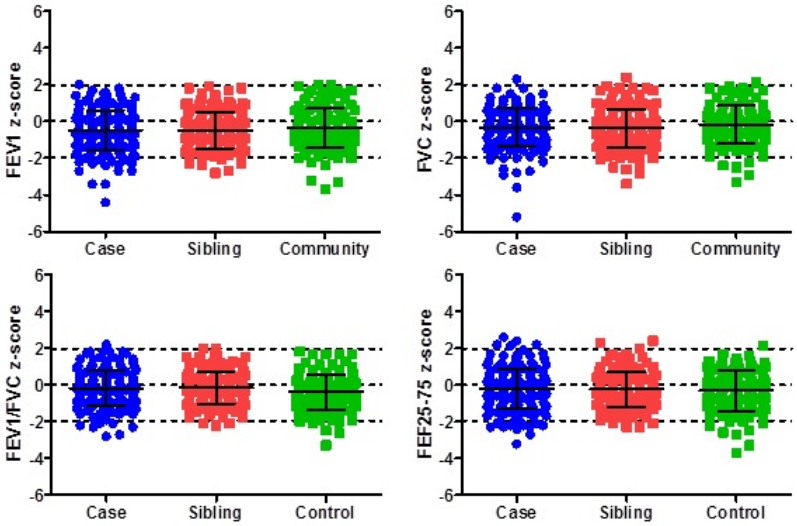


Figure 34: Bee swarm plots illustrating lung function z scores for each of the 3 study groups: cases, siblings and community controls. There is no significant difference in spirometry outcomes between the 3 groups.

	Cases (n=199)	Sibling (n= 140)			Community (n=121)		
	Mean (SD)	Mean (SD)	Unadjusted difference (95% CI) *P value*	Adjusted difference (95% CI) *P value*	Mean (SD)	Unadjusted difference (95% CI) *P value*	Adjusted difference (95% CI) *P value*
FEV_1 (litres)	1.26 (0.3)	1.55 (0.7)	0.30 * (0.2, 0.4) *<0.001*	0.07 * (0.0, 0.1) *0.009*	1.33 (0.34)	0.10 (-0.0, 0.2) *0.07*	0.04 (-0.0, 0.1) *0.12*
FVC (litres)	1.45 (0.3)	1.77 (0.6)	0.33 * (0.2, 0.4) *<0.001*	0.05 (-0.0, 0.1) *0.09*	1.54 (0.4)	0.10 (-0.0, 0.2) *0.07*	0.06 (-0.0, 0.1) *0.054*
FEV_1:FVC	0.88 (0.1)	0.89 (0.1)	0.01 (-0.0, 0.0) *0.30*	0.02 * (0.0, 0.0) *0.012*	0.87 (0.1)	-0.01 (-0.0, 0.0) *0.29*	-0.01 (-0.0, 0.0) *0.54*
FEV_1 Z score	-0.47 (1.1)	-0.48 (1.0)	-0.02 (-0.2, 0.2) *0.88*	-0.11 (-0.4, 0.1) *0.40*	-0.34 (1.1)	0.13 (-0.1, 0.4) *0.30*	0.01 (-0.3, 0.3) *0.94*
FVC Z score	-0.32 (1.0)	-0.38 (1.1)	-0.06 (-0.3, 0.2) *0.61*	-0.21 (-0.5, 0.1) *0.12*	-0.15 (1.1)	0.17 (-0.1, 0.4) *0.17*	0.04 (-0.2, 0.3) *0.77*
FEV_1:FVC Z score	-0.21 (0.9)	-0.15 (0.9)	0.06 (-0.1, 0.3) *0.54*	0.14 (-0.1, 0.4) *0.25*	-0.37 (1.0)	-0.16 (-0.4, 0.1) *0.14*	-0.16 (-0.4, 0.1) *0.21*

Table 37: Results of simple and multivariable linear regression analysis for four main spirometry outcomes across the 3 study groups. The first 3 rows are raw spirometry outcomes, the subsequent 3 rows are z scores based on GLI reference data. Adjusted difference includes sitting height, HIV status, SES and puberty. Age and sex are also included as potential confounders for variables not in z score format. * indicates $p<0.05$.

7.5.5 Predictors of Lung Function in Case Group

I also explored whether factors which differed between the cases at admission could predict poor spirometry outcomes at 7 years post-discharge. There is no difference in spirometry outcomes when comparing degree of wasting or degree of stunting at admission within the case group. Presence of oedema at admission also has no effect on spirometry outcomes. Reported birth weight (i.e. mother reported child to be born "normal or big" or "small" has also no association with spirometry outcomes in the case group. However, age at admission is negatively associated with spirometry outcomes. Additionally, I also explored whether sex, wealth and HIV are factors associated with spirometry outcomes; HIV and sex are significantly associated.

Mean differences for these predictors of poor lung function are presented in **Table 38**.

Potential Predictors	Mean difference FEV_1Z (95% CI)	Mean difference FVCZ (95% CI)	Mean difference FEV1/FVC Z ratio (95% CI)
Stunted at admission (HAZ <-3)	0.09 (-0.1, 0.3)	-0.06 (-0.3, 0.2)	0.07 (-0.2, 0.3)
Wasted at admission (WAZ≤-4)	0.19 (-0.1, 0.5)	-0.10 (-0.4, 0.2)	0.46* (0.2, 0.7)
Oedema at admission	-0.18 (-0.6, 0.2)	-0.14 (-0.5, 0.2)	-0.28 (-0.6, 0.1)
HIV negative vs positive	0.55* (0.2, 0.9)	0.50* (0.2, 0.8)	0.16 (-0.1, 0.4)
Poorest vs Richest SES	-0.10 (-0.4, 0.2)	-0.08 (-0.4, 0.2)	-0.07 (-0.3, 0.1)
Females vs males	-0.31* (-0.6, -0.0)	-0.26 (-0.6, 0.0)	-0.04 (-0.2, 0.1)
Normal vs low birth size	-0.01 (-0.5, 0.5)	0.06 (-0.5, 0.6)	-0.00 (-0.5, 0.5)
Admitted ≤2 yrs vs >2 yrs	-0.47* (0.1, 0.7)	0.32* (0.0, 0.6)	0.08 (-0.2, 0.4)

Table 38: Results of Student t test comparing predictors of poor long-term FEV_1, FVC and FEV_1/FVC z scores in SAM survivors (case group only). * indicates $p<0.05$

7.5.5.1 Lung Function and Age of SAM admission

I would like to explore this outcome further. I hypothesised that those children admitted with malnutrition at an earlier age would likely have more of a deficit in lung function than those admitted at an older age, due to the greater likelihood of disrupted lung development, however that is not the case, hence this warrants further exploration. The mean age of admission of our case group who had technically acceptable spirometry is 2.4 years (n=237). FEV_1 and FVC z scores are significantly associated with age of admission; FEV_1/FVC ratio z score is not affected. As seen in **Table 39**, for each year increment in age of admission, FEV_1 is 0.15 z score's worse, therefore suggesting that being admitted at an older age is indicative of worse lung function 7 years later, the opposite of our hypothesis. Although 0.15 z scores is statistically significant, this is not clinically significant.

When I compared those admitted on or before 2 years and those admitted after with the control group, we see that despite the older admission being more stunted with smaller chest circumference than controls, their spirometry z scores are not significantly different to controls in either age group (see **Table 40**). This is therefore inconclusive.

Lung function measure vs age at admission	**Unadjusted difference** (95% CI) *p value*	**Adjusted difference** (95% CI) ***p value***
FEV_1 z-score	-0.22 * (-0.3, -0.1) *<0.001*	-0.15 * (-0.3, -0.1) *0.004*
FVC z-score	-0.20 * (-0.3, -0.1) *<0.001*	-0.15 * (-0.2, -0.0) *0.004*
FEV_1:FVC z-score	-0.06 (-0.1, 0.0) *0.14*	-0.05 (-0.1, 0.1) *0.36*

Table 39: Simple and multivariable linear regression of lung function z scores against age at admission (years) (cases only). Adjusted difference includes sitting height percentage, HIV status, SES and puberty. * indicates $p<0.05$

	Survivors admitted before or at 24 months (n=106)		**Survivors admitted after 24 months** (n=94)	
	Regress coef. (95% CI)	P value	Regress coef. (95% CI)	P value
HAZ	-0.03 (-0.26, 0.2)	0.81	-0.62 (-0.86, -0.37)	<0.001*
Sitting height %	0.31 (0.01, 0.61)	0.04*	0.58 (0.026, 0.90)	<0.001*
Chest circumference	-0.16 (-1.0, 0.7)	0.71	-1.34 (-2.2, -0.5)	0.003 *
SES	0.05 (-0.2, 0.3)	0.71	-0.05 (-0.3, 0.2)	0.73
FEV_1Z	0.03 (-0.2, 0.3)	0.80	-0.12 (-0.4, 0.2)	0.39
FVC Z	0.09 (-0.2, 0.4)	0.53	-0.10 (-0.4, 0.2)	0.50
FEV_1:FVC Z	-0.02 (-0.3, 0.2)	0.85	-0.01 (-0.3, 0.3)	1.0

Table 40: Results of linear regression analysis comparing growth and spirometry outcomes of those who were admitted before or at 24 months and those who were admitted after 24 months to controls, adjusted for HIV status, SES, puberty and physical disability. Spirometry outcomes are also adjusted for sitting height ratio. Sitting height ratio and chest circumference are also adjusted for age and sex as they are not z score calculations. * indicates $p<0.05$

7.5.6 Summary

Malawian children in this cohort have surprisingly robust lung function compared to African-American data. Characteristics significantly associated with poor spirometry outcomes in this group are:

- HIV positive status
- Low SES
- Female gender

This data suggests that SAM has had little impact on long-term lung function. There is no difference in FEV_1, FVC or FEV_1/FVC z scores between cases and either type of control. SAM survivors who are HIV positive or admitted at an older age (>24 months) have poorer lung function at 7 years-post discharge than other SAM survivors. Those who were more wasted at admission, more stunted at admission, admitted with oedema or reportedly low birth weight have no significant deficit in long-term lung function. The implications of these results are discussed in Chapter 8.

8 Chapter 8: Discussion

This PhD set out to explore the long-term (7-year) outcomes of an episode of SAM, with particular focus on growth, body composition, physical function and lung function. In addition, it explored further risk factors for SAM survivors, such as age at admission, and considered the effects of HIV on the main outcomes. Following a description of the cohort in Chapter 3, the results of these analyses have been presented in Chapter 4-7. In this chapter I discuss the results for each of the main outcomes: growth, body composition, physical function and lung function, and place them in the context of existing knowledge from other studies. I also discuss emerging topics of importance including effects of HIV on the main outcomes and focus on age at admission as an interesting predictor of adverse outcomes following SAM. The chapter finishes with a discussion of the overall picture of adverse outcomes following SAM and the implications of the results for science, programmes and policy.

8.1 Summary of Hypotheses and Results

1. **Initial Hypothesis**: Compared to controls, SAM survivors have impaired linear growth, specifically (a) HAZ (b) shorter leg length but (c) preserved head circumference

   Results: Impaired growth in SAM survivors compared to both sibling and community controls was observed; SAM survivors had (a) lower HAZ than community controls and sibling controls (b) shorter relative leg length and larger sitting height percentage than both types of controls but (c) preserved head circumference. Additionally, cases had lower WAZ than community controls.

2. **Initial Hypothesis**: Compared to sibling controls, linear growth velocity is continuing at normal rates resulting in no catch-up growth since 1 year post-discharge.

   Results: SAM survivors had greater growth velocity than sibling controls, resulting in significant catch-up growth between 1 and 7 years post-discharge, contrary to the original hypothesis.

3. **Initial Hypothesis**: Compared to controls, SAM survivors have an altered body composition, specifically (a) greater proportion of central fat to peripheral fat, (b) a lower proportion of lean mass.

   Results: SAM survivors were observed to have some elements of altered body composition compared to controls; (a) MUAC, calf, waist and hip circumferences indicated that SAM survivors have greater proportion of central to peripheral mass, however skinfold thickness did not suggest that this was as a result of differences in subcutaneous fat distribution, (b) circumference results as well as BIVA results indicate that cases do have a lower proportion of lean mass than controls.

4. **Initial Hypothesis**: Compared to controls, SAM survivors have (a) lower levels of physical activity, (b) lower physical capacity during an exercise test and (c) weaker hand grip strength.

   Results: Compared to controls, SAM survivors were observed to have (a) lower levels of physical activity, however, the sample size was too small to be conclusive; (b) the only evidence of lower physical capacity is their slower HR recovery than community controls (c) they have weaker hand grip strength and lower arm muscle area (AMA).

5. **Initial Hypothesis**: Compared to controls, SAM survivors have impaired lung function (namely reduced FEV_1, FVC and/or FEV_1/FVC ratio z scores). Additionally, those who suffered SAM at a younger age have greater lung function impairments.

   Results: Contrary to all aspects of this hypothesis, SAM survivors had similar FEV1, FVC and FEV1/FVC ratio z scores to controls. Younger SAM sufferers did not have greater lung function impairments; older SAM sufferers actually have significantly poorer lung function than younger admissions (<24 months).

8.2 Growth

25% of community controls were stunted and 21% were underweight; this is high considering that the community controls were intended to be a "healthy" comparison group. Malawi DHS for 2012 presents nutritional status for children under 5 only, showing that 47% of 5 year olds are stunted and 13% are underweight[98]; data on nutritional status of children older than 5 years is not available. The WHO would classify the community control group as having "medium" levels of stunting and "high" levels of underweight; sibling controls would classify as both high stunting (33%) and high underweight (23%), and ex-malnourished cases would be classified as "very high" for both (41% stunting, 33% underweight)[259].

These results show that SAM survivors are more likely to be underweight and stunted after recovery than children in their community, even as long as 7 years post-discharge. Being underweight or wasted is associated with short-term nutrition deprivation whereas stunting is associated with chronic undernutrition[260]. Results showing that ex-malnourished cases have a significantly lower WAZ than community controls but not significantly different to sibling controls suggests that access to nutrition remains low for case children at a household level. SAM survivors are more stunted than both their community and sibling controls suggesting that this is not a household issue but directly related to the SAM admission. It was hypothesized that this would be the case based on findings by Kerac et al., that case children were significantly more stunted than their siblings at 1 year post-discharge[97]. Richard et al., also found that previously wasted children were more likely to be stunted at 2 year of age using longitudinal data from 8 cohort studies[85]. It is also thought that it is difficult to catch-up stunting after the first "1000 days" of life (conception to two years)[92], although this has been disputed[93]. The link between wasting and stunting is complex and has been a topic of some recent discussion[261]. I have found that children who have been severely wasted are more likely to be stunted in late childhood. Stunting has known long-term consequences; namely poor cognitive function, lower earning potential and poor reproductive health[84].

The stunting of SAM survivors can be accounted for largely by shorter limbs than community and sibling controls. The proportion of height accounted for by sitting height is greater in SAM survivors than controls, suggesting that torso growth has been preserved and limbs compromised, potentially as a result of the SAM episode. Leg length in relation to height is considered an indicator of the quality of the environment for growth during infancy and childhood[113]. These results could indicate that SAM children undertake "brain-sparing growth" or have adapted a "thrifty phenotype" where growth of vital organs has been spared and less vital growth, such as limb length, compromised [29, 30]. This is supported by the preservation of head circumference of SAM survivors compared to controls. Birth weight could be a significant confounder in this study, which we do not have accurate data for, however only 9% of surviving cases were reported

to be small or very small at birth. The pattern of growth seen by these SAM survivors may have significant implications for adult health; sitting height percentage has been associated with cardiovascular disease (CVD) and diabetes in adulthood, and short limb length is associated with higher adult blood pressure[121, 123, 124]. Additionally, evidence from LBW cohorts (Helsinki cohort and the Hertfordshire studies) found that low WAZ and BAZ at 1 year of age were more strongly associated with heart disease than LBW or low WHZ at birth[262]. This suggests that postnatal nutrition plays a larger role in risk of heart disease than prenatal nutrition, although it is important to remember that birth weight is only a crude measure of foetal nutrition. This study therefore adds some preliminary evidence to the theory that SAM in childhood may be contributing to the inexplicably high levels of chronic diseases in developing countries[95].

It's not all bad news for this cohort. There is some evidence to suggest that the growth pattern of these SAM survivors might be changing. Kerac et al., saw a large improvement in WAZ between discharge and 1 year post-discharge, but almost no change in HAZ. In the following 6 years, we have found that the SAM survivors have made a very small improvement in WAZ but large improvements in HAZ. In order to minimise effects of "regression to the mean", we compared the growth of cases to sibling controls between 1 and 7 years post-discharge and found that cases are indeed catching up HAZ at a steeper rate than their sibling controls. The gap in HAZ between the SAM survivors and their siblings is closing. There has been much conflicting data regarding the possibility of catch-up growth in stunted children, partly due to studies using different definitions of "catch up growth" (e.g. catching up to community levels or global levels) and different study end points[103, 104, 107]. One recent study found that both growth faltering and catch up growth could occur between ages of 8 and 15 year, concluding that child growth and development is a dynamic process which offers continued opportunities and sustained risks for children after the first 1000 days[263]. The same study also found that those children who caught up their HAZ to their community norm suffered less of a cognitive deficit than those who didn't catch up, although this is not consistent across all studies[264]. This suggests that there may be opportunity to mitigate some of the long-term effects of stunting if it is somewhat reversed by adulthood; i.e. there are opportunities for intervention even after the first "1000 days". Our study suggests that the Malawi SAM survivors are on track to catch-up some of their linear growth; biologically there is also still an opportunity for reversal of sitting height percentage as well as growth plates are yet to have fused[93]. These results, along with the findings from a few other African cohorts, challenge the conventional view in the literature that catch-up growth after early childhood is difficult and seldom observed [107, 265, 266]. Some papers offer emerging evidence that puberty provides an even greater window of opportunity for catch up growth[93, 267], although Adair et al., found puberty to also be a period of missed opportunity where growth faltering can occur and gains in height-for-age during childhood can be lost[103]. Whether these SAM survivors will

continue to be more stunted than their community and have adverse body proportions after adolescence is yet to be seen.

Exploring risk factors which predict adverse long-term growth outcomes offers some opportunities for considering potential interventions. Kerac et al., found disability and HIV to be significant risk factors in post-SAM survival[97]. They also found that children admitted with marasmus (rather than kwashiorkor) had worse WAZ and HAZ both at admission and at 1 year post-discharge. This study found that HIV continues to be a risk factor for poor outcomes, namely survival, stunting and short relative leg length. This study also found that marasmus survivors continue to have significantly lower WAZ than kwashiorkor survivors however their HAZ is no longer significantly different, and their sitting height percentage and head circumference are also no different. It seems therefore that marasmus survivors are still more prone to being underweight but otherwise have similar growth to kwashiorkor survivors. Survivor bias cannot be the explanation for the continued differences in WAZ as risk of death during treatment was also higher in non-oedematous children. There are no apparent long-term effects on growth relating to how quickly patients recover from SAM.

We found that the most significant risk factor for long-term growth impairments was age at admission. These results suggest that the older a child is at admission, the worse their HAZ, MUAC and BAZ will be at 7 years post-discharge. It appears that as children get older, possibly as they move beyond the "first 1000 days", their growth becomes canalized and less able to make good a severe insult. This emphasises the need for early intervention and active case-finding in young children so that SAM can be prevented as they grow older. In this aspect, our data supports the concept that first 1000 days is an integral time for intervention, as the capacity for recovery is greater in those who has SAM at <2 years of age. These results found that catch-up growth is possible after the first "1000 days" however if the insult occurs after the first 1000 days, the catch-up ability is much more limited. Nutrition interventions that go beyond the first 1000 days in order to prevent SAM in children older than 2 years could therefore also help to reduce ultimate levels of stunting. For children older than 2 years who do suffer an episode of SAM, it could be argued that there is a greater need for nutrition interventions in this group of survivors as their capacity to recover without intervention is more limited, although we have seen that some catch-up growth is possible. The most obvious nutrition intervention for children >2 years, particularly in adolescence, is effective school feeding programmes.

In summary, this study suggests that SAM survivors are at higher risk of being stunted, but that some linear catch-up growth is possible. Although it is clear that prevention is far better than cure, interventions, such as school feeding, which capitalise on this catch-up ability and promote linear growth, especially during adolescence, could make use of the "second window of opportunity" to

reduce stunting, correct adverse body proportions and thereby potentially reduce subsequent chronic diseases risks in adulthood.

8.3 Body Composition

SAM survivors were observed to have some elements of adverse body composition compared to controls, including lower peripheral mass, a larger waist circumference, a smaller hip circumference and significantly less lean mass, as measured by BIVA. MUAC, calf, waist and hip circumferences indicated that SAM survivors have greater proportion of central to peripheral mass, however skinfold thickness did not suggest that this was as a result of differences in subcutaneous fat distribution. Waist/hip ratio indicates a small difference in gluteo-femoral to core fat distribution. Skinfold thickness specifically compares limb subcutaneous fat to core subcutaneous fat. The lack of difference between cases and controls for this outcome could be because proportions of FM are likely to be very low for majority of cases and controls in this population, although comparison of body composition outcomes to other populations is made difficult by the lack of relevant reference data. Had cases and controls had the opportunity to accumulate some FM, perhaps they we would see differential distribution across limbs and core. This will likely only be a relevant consideration once Sub-Saharan Africa makes the nutrition transition associated with economic development and urbanisation. Another explanation is that skinfold thickness ratios have poor statistical validity; it has been said that as the relationship between limb and torso skinfolds is not linear, dividing one skinfold by another is a problematic way of assessing relative fat distribution[219].

Although BIA cannot measure distribution of fat mass, it does give an indication of overall FM levels which appear to be similar across the study groups. The reduction in lean mass of the cases (even after adjusting for their shorter height) but preservation of fat mass levels is a similar pattern seen in childhood of low birth weight infants[67]. Studies of Indian infants have found infant weight gain is positively associated with later lean mass but not fat mass, indicating that poor infant growth constrains later lean mass[268]. Disruptions to lean mass in early life are predicted to reduce the capacity for homeostasis, and increase the risk of chronic degenerative diseases in adulthood[96]. Additionally, peripheral fat and peripheral lean mass, which appear diminished in these SAM-survivors, are thought to be protective again CVD, particularly hip circumference [132, 269]. Peripheral lean mass also has implications for physical function. Although circumferences suggest that siblings have more lean mass, particularly peripheral lean mass, than cases, BIA data suggests no difference in amount of lean mass, fluid and fat mass for these two groups. Either household levels of undernutrition mean there is no difference in levels

of lean mass between cases and siblings, or larger standard deviation of BIA readings, perhaps due to larger age range in siblings means that although r/h of siblings is lower on average, it does not meet the cut off for significance.

When considering the results of BIVA, we can summarise that, as mentioned in **Chapter 5**, long vectors with a small phase angle (increased R with decreased Xc) are associated with malnutrition, whereas short vectors with a small phase angle (decreased R with decreased Xc) are associated with fluid overload[231]. This suggests that our cases are still relatively more malnourished and have less fluid than community controls. Lower levels of fluid can be explained by cases having lower proportion of "high hydration" tissues than controls[270]. Muscle mass is a high hydration tissue, and as cases have less lean mass, smaller MUAC and smaller calf circumference, this likely explains their lower fluid levels. We also observed sexual dimorphism with greater lean mass in boys and greater fat mass in girls; this is in line with a multitude of previous studies [67, 271-273]. When stratifying analysis of cases vs controls by sex some differences in body composition are only present when comparing control girls to case girls. Although a rough measure of puberty was adjusted for, I believe this reflects the difference in body composition and fat accumulation which happens earlier in girls than boys[274].

As well as less lean mass and less fluid, BIA tells us that cases have a significantly lower phase angle (PA) than community controls. Phase angle is thought to be a measure of cell health, where higher values reflect higher cellularity, better cell membrane integrity and better cell function, as well being as an indicator of general health and nutritional status [235, 275, 276]. Some studies have found it useful in identifying malnutrition, particularly in discriminating between different forms of underweight with regard to low fat and low lean mass[235, 277]**.** However, some studies have found PA to be unreliable in identifying malnutrition and morbidity depending on the "cut-off" value used[278, 279]. There is currently no universally accepted cut off value for "low PA", particularly in children. Kyle et al., suggests PA of <5 in men and <4.6 in women as cut off for high nutritional risk in adults. One study of PA in a small number of hospitalised children in Japan suggested that most malnourished children have a PA <3.2[280]. The mean phase angle for each of our study groups (5.0, 5.1 and 5.1) is therefore relatively "healthy". Only four children in this sample had a PA<3.2, two were cases and 2 were community controls.

Lastly, when considering predictors of adverse body composition in SAM survivors, the most notable factor for discussion is marasmus vs kwashiorkor. The results suggest that those who were admitted with marasmus now have significantly less fluid and less lean mass and lower PA than controls, whereas those who were admitted with kwashiorkor have similar fluid and lean mass to controls. Kwashiorkor survivors have therefore attained a "normal" body composition

relative to their community whereas marasmus survivors have not. This correlates with significantly lower WAZ of marasmus survivors. Could it be possible that oedema develops in some severely malnourished children as a mechanism to protect against loss of lean mass and resultant long-term implications? Or is kwashiorkor an "early warning system" is some children, in which case we can infer that earlier intervention results in fewer long-term implications and should therefore be policy norm. Why some children develop kwashiorkor and others marasmus is still not known and warrants further study.

In summary, this study suggests that SAM survivors have less lean mass than community controls, and less peripheral lean mass than both community and sibling controls. Peripheral mass is protective against some NCDs and has implications for physical function. Cases also have lower phase angle than community controls which is indicative of nutritional and morbidity risk, although very few children have PA to qualify them as "malnourished". Marasmus survivors are at particular risk of long-term adverse body composition.

8.4 Physical Function

Compared to controls, SAM survivors were observed to have lower levels of physical activity however sample size was too small to be conclusive; cases had poorer HR recovery from the exercise test than community control, but not sibling controls; and cases had weaker hand grip strength and lower arm muscle area (AMA).

Community controls age 8-9 years had mean hand grip strength of 11.9kg, this was significantly lower than mean grip strength of 8-9 year olds from one study in USA (17.5kg)[281]. Hand grip strength of cases was even lower. A number of other studies have developed reference ranges for hand grip strength, however they use a variety of different units including newtons and kilopascals, making comparison difficult[142, 282]. For AMA, Benefice et al., found 10-12 year olds Senegalese school children had AMA of 18.4cm^2; our community controls have a larger mean AMA of 23.3cm^2 in this age group, despite having a similar height[142]. Perhaps due to changes in nutrition across Africa since 1992, the Senegalese data is now out of date. Deficiencies in grip strength and AMA observed in case children compared to controls is not surprising considering deficits in HAZ, WAZ and lean mass also observed. However it is still important to note that these deficits in growth are severe enough to have functional implications. Additionally weak hand grip strength is associated with all-cause early mortality, nutritional risk and risk of NCDs, and it is being promoted as a non-invasive indicator of mortality risk[159]. These results have also highlighted the importance of healthy weight gain for SAM survivors, as better WAZ was associated with better muscle strength.

Regarding physical activity, it is disappointing that a combination of logistical issues and non-compliance resulted in a small sample size, with a large standard deviation. However differences in standard deviation are large in biological terms and suggest cases may have lower physical activity levels than controls. Mean steps per day of the cohort overall were roughly in line with European levels which estimate 8-10 year old children to take 12,000 to 16,000 steps/day (1000-1330 steps per hour)[283]. European girls are thought to take fewer steps than boys which is also observed in our case data; European teenagers take fewer steps than children, although this doesn't seem to hold true for our Malawian data. A further study is required to investigate whether there is a true deficit in long-term physical activity is SAM survivors, as this could have serious implications for NCDs in adulthood, especially as the nutrition transition of the country progresses. This study has been able to identify the required sample size of around 300 per group for a future study.

For physical capacity, cases completed similar minutes of the exercise test as sibling and community controls. Their maximum heart rate was not significantly different to controls but their heart rate was significantly slower to recover than community controls. This may suggest a small difference in physical capacity between cases and community controls, although they do not have the marked drop in oxygen saturation or raised heart rate seen in cystic fibrosis patients for whom the exercise test was originally designed[151]. I must also acknowledge that the operators of the iStep test were not blinded to the study group status of the children, hence potentially differential encouragement from operators could have affected the time at which the children decided they were too tired to continue.

Based on the range of evidence for physical function outcomes discussed here, it seems likely that SAM survivors have worse long-term physical function compared to children in their community. Considering this and the known anabolic effect of exercise on muscle growth, an intervention which combines both nutrition for better accumulation of weight, particularly lean mass, and physical activity for building strength and physical capacity, could have large benefits for the long-term health of SAM-survivors. A few studies have considered physical activity interventions during immediate SAM recovery, with promising results. Viteri et al., explored the role that exercise played on the growth of malnourished weanling rats, discovering that physically active animals grew more in length and weight than their inactive counterparts in both the well-nourished and malnourished groups[284]. Human studies have also concluded that malnourished children recovered faster in centres with playgrounds than in smaller centres, and one study found children recovering from oedematous SAM in Guatemala who were encouraged to do "active" play 6 weeks post-SAM showed similar clinical improvement to the control group but had significantly greater linear growth after 5 weeks of intervention; they also grew more in lean body mass as well as having higher creatinine-height index (0.97 ±0.12, vs 0.89 ±0.09, $P < 0.05$)

indicating greater muscle mass and protein repletion[154, 285]. These studies suggest that moderate but persistent exercise enhance catch-up growth post-SAM. Authors suggest that the mechanism may be mediated by endocrine factors that promote the growth of long bones, such as growth hormone and insulin-like growth factors, which are stimulated by physical exercise. Others have argued that very little exercise is required to stimulate bone growth[154]. Lean mass, which is significantly lower in cases, is not only an outcome of physical function but also an important predictor of physical work capacity in later life and could be the likely explanation for the deficiencies in physical function seen in these SAM-survivors[286]Based on our evidence that SAM survivors are more stunted, have weaker hand grip strength and potentially lower physical activity levels and lower physical capacity, an intervention which encourages exercise in SAM survivors could be beneficial in a variety of aspects.

Regarding risk factors at admission, those who were admitted with oedema have similar handgrip strength and similar physical capacity to those admitted without oedema, but oedematous admissions have larger AMA than marasmic admissions, at follow-up. This result correlates with higher levels of lean mass observed in kwashiorkor survivors by BIA data. This suggests that it is not always quantity of muscle that affects muscle strength; perhaps both quantity and quality are important. Also interesting is the lack of impact observed by HIV status on these functional outcomes. Most children who are known to be HIV positive (84%) reported taking treatment, either Cotrimoxazole or antiretroviral therapy (ARVs), although some did report problems with taking their medication; perhaps this high coverage of treatment is mitigating the functional impacts of HIV; although the same cannot be said for lung function.

8.5 Lung Function

Contrary to our hypothesis, no evidence was found that suggests SAM survivors have poor lung function compared to sibling or community controls. The quality of the results is unlikely to be an issue with standard deviation of z score values very close to 1, and with overall fail rate for spirometry results of 12% (64/532), which is in line with results from studies conducted in expert settings in developed countries[255].

The mean spirometry z scores achieved by this cohort are within 0.5 z scores of the reference values for black children in USA. Malawian boys have especially robust results with mean FEV_1Z of -0.31 (95% CI -0.4 to -0.2) and mean FVCZ of -0.17 (95% CI -0.3 to -0.0). It is difficult to compare our results to other Malawian studies due to use of different reference ranges, however our results appear closer to the reference range used than results in other studies. A study of children with sickle cell disease in Blantyre, Malawi (median age 11.7 years) found mean z scores of −1.64 (95% CI −2.04 to −1.23) for FEV_1 and −1.49 (95% CI −1.90 to −1.09) for

FVC[287], based on an international reference for Black children by Wang et al[288]; although these were not healthy children. A study of 514 Malawian primary school children found FEV_1 z score of -1.0 and FVC of -0.7 using a European reference range with a scaling factor for black ethnicity[253]. They state that their z scores are slightly lower than spirometry results found in Sudanese and Libyan children, but similar to Nigerian, Tanzanian and Jamaican children[289-293]. Their results are similar, although slightly worse than those found in our cohort; however the use of a standard scaling factor for black ethnicity (-1.3 z scores) cannot be as accurate as comparing to actual data from black children. I chose not to use the reference data generated from the Malawian primary school study as numbers are relatively small, the children's age is rounded to a whole number and the reference values are generated only on height, rather than height and age.

The apparent absence of any impact from SAM on spirometry outcomes is surprising, particularly given the deficits in WAZ, HAZ and hand grip strength seen in the case group compared to the community controls. Our result suggest that while the overall volume of the lungs may be smaller in SAM survivors, it is proportional to their body size (height) and there is no evidence of lung function quality defects. Evidence from animal models, as well as some human studies in India, show that postnatal malnutrition can disturb lung function in the form of qualitative change beyond an effect on lung size [176, 294, 295]. Animal models can be conclusive in stating that early postnatal malnutrition affects lung development, and this has impacts on lung function; however human studies can only conclude that there is a correlation between lung function and current nutritional status. Our data also confirms this. However, the reason for the correlation cannot be deduced from these results, and interestingly we have observed that an episode of SAM with associated stunting and muscle weakness is not enough to have an impact on lung function. Perhaps the correlation of WAZ and HAZ with lung function exists due to confounding with prenatal malnutrition, very early postnatal malnutrition or other poor environmental factors including recurrent infections. Our evidence suggests that a severe nutritional insult between the ages of 6 months and 2 years does not have qualitative effects on lung development beyond effects on lung size. Even children admitted after 2 years of age, although sustaining a greater impact on growth and lower lung function, their lung function is still not significantly lower than control children, after adjusting for HIV (p=0.24 and 0.20 for FEV_1Z and FVCZ respectively).

A study of Indian children (6-12 years) found that although weight-for-age affected lung function (peak expiratory flow rate) height-for-age had little effect. They hypothesise that low WAZ affects lung function due to its negative effect on muscle function, but that past or chronic malnutrition does not affect lung function even though it affects somatic growth[175]. Our evidence suggests that SAM and subsequent stunting have not had a qualitative impact of lung function which could be due to the preservation of sitting height among stunted children. These

SAM survivors, despite their significantly reduced HAZ, have similar sitting height to controls and significantly larger sitting height percentage. Additionally, FEV_1 and FVC z scores are positively associated with sitting height percentage. It would be interesting to see whether catch-up in HAZ, presumably limb growth, which our SAM survivors are currently undertaking, results in decreased spirometry z scores. This knowledge would help unpick the complexities of stunting, lung size and lung quality.

When exploring predictors of poor lung function, we see that HIV has a significant impact. There is emerging data for HIV positive adults suggesting an increased prevalence of chronic lung disease, including both chronic airflow obstruction and restrictive lung disease [296-298]. Studies have also shown odds of airflow obstruction were increased >3-fold among HIV-infected adult individuals in USA, although smoking and drug use are contributing factors in these populations[299]. Very few studies have considered effect of HIV on lung function in children. Despite the significant impact of HIV on lung function in this cohort, we did not observe any impact of HIV on physical function. We did find that although there is no association between having a history of pneumonia and HIV status, the odds of having a history of TB are 8 times higher in HIV positive children than negative children (95% CI 3.2 to 19.7); additionally those with a history of TB have significantly lower FEV_1 and FVC z scores, although those with a history of TB were excluded from final analysis. HIV positive children are also significantly more stunted than HIV negative children although there is no difference in sitting height percentage between HIV positive and HIV negative groups. It could be a combination of repeat infections, such as TB, and chronic malnutrition due to greater calorie requirements that have already reduced lung function in HIV positive children, despite widespread management with medication.

The last significant variable associated with lung function to discuss is sex. Females have significantly lower FEV_1 and FVC than males in this study, suggesting a more restrictive lung function pattern. I explored whether this was a result of differences in adherence to the reference data between the sexes. As lung function increases linearly with age until the adolescent growth spurt at about age 10 years in girls and 12 in boys[288], it could be that delayed puberty known to occur in malnourished populations could result in a divergence from the reference data around the age of 10 years in females[300]. This would mean the difference between the sexes would only become apparent around the age of 10, and this is not the case as presented in Chapter 7. I also explored lung function, sex and body composition as studies looking at older European adults have found that a larger proportion of FM is associated with worse lung function and more lean mass is associated with better lung function[257]. One paper looking at cystic fibrosis in children found that more lean mass meant better lung function; they also found that more fat mass meant better lung function but not significantly so[256]. Males in our cohort have more lean mass than girls (based on LMI and BIVA), and less fat mass, particularly peripheral fat mass. LMI is

positively associated with FEV_1Z and FVCZ whereas fat mass is negatively associated with FEV_1/FVC z ratio; hence this could be an explanation for differences in spirometry between the sexes.

Zverev et al., who conducted the study of spirometry in primary school children in Malawi (discussed above) also found significantly lower FEV_1 and FVC in females than in males, however they do not discuss why this might be[253]. The other option for this difference is differential exposure to household air pollution. There is evidence that household air pollution can lead to respiratory infections and chronic lung disease; women and children are particularly susceptible to the toxic effects of pollution and are exposed to the highest concentrations[301]. Whether girls in Malawi as young as 9 years have greater exposure to cooking smoke than boys the same age is not known; our questionnaire asked about cooking smoke in the home but it did not ask specifically who helps with the cooking or whether girls experience greater exposure than boys. However, this is another possible explanation for spirometry sex differences. Further study is certainly needed in this area, as well as further exploration of the effects of HIV on lung function in children.

8.6 HIV

HIV is an important potential confounder and/or effect modifier in this research project. HIV increases ones risk of SAM (the exposure) and is likely to increase ones risk of some adverse outcomes, such as stunting for example[302, 303]. This cohort, as well as others, have seen that severely malnourished children with HIV infection have an increased risk of mortality compared with their HIV-uninfected counterparts[97, 304]. The extension of the time-to-death curves, stratified by HIV status (Figure 5b, Chapter 3), highlight the significant adverse impact of HIV on post-SAM survival. Probability of death in the HIV negative patients plateaus around 6 months after admission whereas the probability of death in the HIV positive group doesn't begin to plateau until around 2 years post-admission, and even then it continues to rise very gently, even as long as 8 years post-admission. The risk of death in the HIV positive group is also made evident by the change in proportion of HIV positive children in the sample over time since admission (see **Figure 35**).

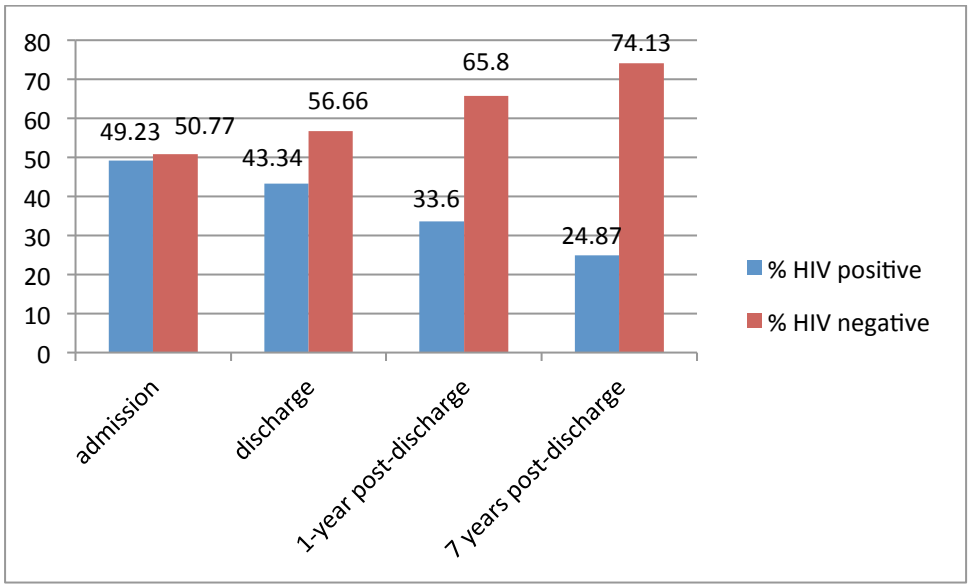


Figure 35: Bar graph showing percent HIV positive and HIV negative children in the case group of the cohort from ward admission to latest follow-up 7 years post-discharge.

A 2009 study has considered the differences between HIV positive and negative children during SAM recovery and 4 months post-SAM treatment; they found increased mortality in the HIV infected group during treatment, but for those who survived there was no difference in weight gain or subscapular and triceps skinfolds during nutrition rehabilitation, nor weight-for-height z score or MUAC at follow-up[305]. Our results concur with these findings as I observed no significant difference in WAZ, BAZ, MUAC, calf, hip and waist circumference between HIV positive and HIV negative SAM survivors 7-years post-discharge. I also found no difference in skinfold thickness, skinfold thickness ratio, fat mass, AMA, hand grip strength and physical capacity for these 2 groups. As already mentioned, most children who are known to be HIV positive in this cohort (n=104) reported taking treatment, either Cotrimoxazole (COT) (n=11) or antiretroviral therapy (ARVs) (n=76). Six had never taken either medication and some did report problems with taking their medication (n=7). There was no difference in HAZ between those taking COT only and those taking ARVs. However, I have observed some important effects of HIV despite the high coverage of treatment, namely greater stunting, less lean mass as measured by BIVA, and worse lung function (more restrictive pattern). HIV positive SAM survivors also reported more difficulty seeing than HIV negative SAM survivors, but no difference in reports of difficulty hearing or walking.

When considering the impact of HIV across the groups, not just in SAM survivors, the HIV positive sibling controls have some of the worst outcomes, resulting in a significant difference in HAZ, LMI, BIVA, FEV_1Z, FVCZ and greater difficulty seeing and hearing than HIV negative children. The HIV positive sibling controls, although numbers were low (n=9), have lower HAZ

and LMI than HIV positive case children. This could be an indication of survivor bias; if survival of HIV positive children during SAM treatment was to improve, we may see even greater differences in long-term stunting and lack of lean mass among survivors.

8.7 Kwashiorkor vs Marasmas

This study has found that kwashiorkor survivors (those who had oedema at admission) generally have better long-term outcomes than those who didn't have oedema at admission. These results found that kwashiorkor sufferers have better survival, significantly higher WAZ, BAZ, lean mass, lower R/h, larger calf circumference and larger AMA than marasmic survivors at follow-up. Differences in body composition between kwashiorkor and marasmic survivors are particularly pronounced; kwashiorkor survivors have no deficits in body composition compared to controls whereas marasmic survivors still have less lean mass, higher R/h and smaller calf circumference than controls. Handgrip strength, physical capacity and lung function were not different between kwashiorkor and marasmic survivors at follow-up.

These results are a little surprising as kwashiorkor is usually found to have a higher case fatality rate than marasmus in many areas[11]. I found only one study that had compared outcomes for marasmus vs kwashiorkor at follow-up: a study in Barbados found no difference in long-term IQ between marasmus and kwashiorkor survivors, both were equally lower than community controls at 11 years of age[306].

Deciphering the long-term differences between kwashiorkor and marasmus survivors found in this study is difficult due to the ongoing knowledge gaps around the pathogenesis of kwashiorkor. Ever since Cicely Williams described the entity known as kwashiorkor in 1935 while working in west Africa, there has been much debate on its causes[11, 306]. Some have suggested that kwashiorkor is related to low dietary protein and/or antioxidant intake; Manary et al (2000) found increased oxidative stress in kwashiorkor sufferers compared to marasmus, suggesting that oxidative damage to proteins and other biologic targets plays a role in the clinical manifestations of kwashiorkor[307]. However a study in 2005 examined the impact of an antioxidant cocktail as a possible preventive treatment for kwashiorkor in children in Malawi and found no protective effect suggesting that antioxidants might not play a central decisive role as originally proposed[308]. Another more recent theory is that differences in gut microbiome may be a causal factor in kwashiorkor development suggested by a study that transplanted faecal matter from kwashiorkor patients into mice and found that this combined with a low protein diet saw marked weight loss and perturbations in amino acid and carbohydrate metabolism[309].

The results described in this thesis suggest that kwashiorkor is protective against some of the long-term adverse outcomes of SAM; WAZ was not as low for kwashiorkor children at admission and this maintenance of weight seems to have protected against subsequent low WAZ, low lean

mass and low calf circumference and arm muscle in later years. We should remember that the bar for admission is much lower for kwashiorkor patients than marasmic patients as presence of any oedema results in admission regardless of WHZ or MUAC qualifications. I believe the most likely explanations for the better outcomes of kwashiorkor survivors is that the presence of oedema worked as an "early warning system" for SAM suffers in Malawi where treatment was initiated before further wasting was encountered. It is important to note that oedema was much more prevalent than wasting in this cohort; at admission 70% (697/1006) of the group had oedema, at the 7 year follow-up 83% (265/320) of the survivors had oedema at admission. The definitions of kwashiorkor and SAM could also be affecting these results as bilateral oedema can be seen in other acute illnesses besides SAM which could have resulted in some misdiagnoses of SAM.

If oedema did indeed result in earlier treatment and subsequent better long-term outcomes, this supports further advocacy for initiation of earlier SAM treatment for all patients, not just those with kwashiorkor, in order to prevent some of the long-term effects.

8.8 Age at Admission

Based on existing evidence for the DOHaD hypothesis, it is infants who are undertaking a large amount of vital and irreversible development, that are more likely to be affected by a nutritional insult than older children. We know that the window of "phenotypic plasticity", i.e. the ability of a single genotype to produce more than one alternative form of morphology, physiological state, and/or behaviour in response to environmental conditions, is a finite period[79]. Plant and animal studies have shown that the window for phenotypic adaption to the environment varies greatly across species and can either be very narrow or very wide[80, 310, 311]. How large the window is in humans is not known; as humans are born at a relatively advanced state in development, it could be that the plasticity period which influences later health stops relatively close to birth[81]. Additionally, the "first 1000 days" concept suggests that nutritional deficits, specifically stunting, can accumulate before the 2nd birthday, and after which point HAZ is relative fixed, neither catching up nor faltering more[92]. Age of insult therefore seems a highly relevant factor when exploring the long-term effects of SAM; it seems likely that those who suffered SAM at an earlier age (i.e. before 2 years), suffered greater disruption to their development and are more likely to have adopted phenotypic changes. I therefore explored effects of age of SAM insult on each of the long-term outcomes.

The median age of admission for SAM survivors in this study was 24 months (range 6-168 months). Although these results have shown that SAM does have long-term effects on a variety of outcomes, I did not observe an increase in detrimental effects in those admitted <2 years of age, contrary to existing theories. There was no difference between those admitted before they were 2

years and those admitted after on long-term WAZ, sitting height percentage, head circumference, waist, hip and calf circumference, skinfold thickness ratio, AMA, hand grip or time completed of exercise test. Those admitted >2 years are more likely to be HIV positive and more likely to have a physical disability; hence these factors were adjusted for as potential confounders during analysis. Older admissions also started with greater levels of stunting at admission. It is less common for children >2 years to be admitted with SAM hence these children are likely to have had some additional complications.

I did however observe that children who were admitted with SAM >2 years had some greater deficits in growth, lean mass and lung function. HAZ, BAZ, MUAC, LMI, FEV_1Z and FVCZ are significantly smaller in children admitted at an older age, and R/H is significantly larger in older admissions. My results therefore found that children admitted when they are older are more likely to be more stunted on admission and for every month increase in age at admission they are likely to be 0.02 z scores more stunted at follow up ($p<0.001$), regardless of how stunted they were at admission. Mortality results also suggest that older admissions are more likely to die than younger admission; although this is a U-shaped relationship with very young admissions (<1 year) also at greater risk of mortality. When compared with controls, the difference in HAZ and lean mass is only significantly lower in those admitted >2 years of age, suggesting that SAM admissions <2 years have caught up their HAZ and lean mass to community levels by 7 years post-discharge. Richard et al., in their meta-analyses also found that older children have less catch-up than younger children, but because their study end-point was the same age for all children rather than a set number of years since the insult they could not conclude whether this was related to the children's catch-up ability or the fact that older children had had less time to catch up.

Although my finding that older admissions have some more detrimental outcomes than younger ones is contrary to my original hypothesis, it does confirm a number of other existing theories. The "First 1000 days" theory states that most stunting is accumulated during conception to 2 years; this is therefore an important period of plasticity for growth and development in which good nutrition is paramount. These results concur with the high plasticity of the first 1000 days period, however with more of an emphasis on opportunity for catch-up rather than opportunity for insult. In contrast, those acquiring SAM in later childhood appear to have minimal plasticity and therefore less capacity for rectifying a poorer quality phenotype. Although the older admissions began with a lower HAZ than younger ones and have undertaken some catch-up growth, they have caught up less height than that gained by the "already relatively taller" younger admissions. These results emphasise the need for early nutritional intervention and active case-finding of moderate acute malnutrition (MAM) and SAM in younger children, to give them the best chance for full recovery. If we miss this window, the chances of long-term growth, body composition and

lung function deficits are increased. Interventions which particularly identify and treat malnutrition in the first 6 months of life could result in significantly fewer stunted children and adults. Identification and treatment of malnutrition in infants is currently lacking in evidence[88]. Additionally, sanitation, health and nutrition interventions that go beyond the first 1000 days and strive to prevent acute malnutrition is children > 2 years should also be a priority for improving long-term outcomes.

8.9 The Overall Picture

The aim of this study was to explore long-term (7 year) outcomes of severe acute malnutrition on growth, body composition, and functional outcomes in children. The results suggest that there are long-term adverse effects of an episode of SAM, particularly with regard to growth, body composition and physical strength. There are few studies that have considered post-SAM outcomes, particularly for longer than 18 months and using the current definitions of SAM, as concluded by a recent literature review[86]. Our study shows that SAM survivors continue to be at slightly higher risk of mortality many years after admission; the time-to-death curves suggest that the majority of mortality risk for SAM survivors in during the first 2-years post-admission; the probability of death has now plateaued for the group of survivors as a whole but it may still be rising very slightly in the HIV positive group.

However, there are also some signs of recovery, for example in their growth. There is some evidence to suggest that their growth pattern might be changing. Kerac et al., saw a large improvement in mean WAZ between discharge and 1 year post-discharge, but almost no change in HAZ[97]. In the following 6 years, we have found that the SAM survivors have made a very small improvement in WAZ but large improvements in HAZ. We compared the growth of cases to sibling controls between 1 and 7 years post-discharge and found that cases are indeed catching-up HAZ at a steeper rate than their sibling controls. These results also suggest that SAM-survivors do not have lasting disruption to all of their major body systems as sitting height, lung function and head circumference are not significantly different to sibling or community controls. It therefore appears that DOHaD does not affect SAM-survivors in the same way seen in low birth weight infants[29]. Although it is also worth noting that community controls are not so healthy themselves: they have higher number of overall hospital admissions than cases. This likely reflects the high levels of morbidity in the community and may be diminishing what we are able to see in terms of the long-term effects of SAM.

Another reason why SAM survivors may have different long-term outcomes to LWB infants might be because this group of SAM survivors have remained thin. The DOHaD theory explains

that LBW infants are susceptible to weight gain once exposed to a higher fat diet, and additionally, they have a diminished ability to cope with the heightened "metabolic load"[126, 312]. This is not currently a consideration for SAM survivors in this cohort and may be the reason why their vital organ function, such as lung function, appears unaffected. However, gain in weight is a strong possibility for this group, based on the current trends in many African countries towards escalating prevalence of obesity and sedentary behaviour, as well as imported unhealthy foods[313, 314]. As economic development proceeds, these SAM survivors may be exposed to a higher 'metabolic load' in adulthood due to increasing BMI, exposing their poorer homeostatic capacity[312].

Although weight gain since discharge has been minimal in these SAM survivors, height gain has been significant. The catch-up growth seen in this cohort suggests that the "first 1000 days" is not the only time that reversal of nutritional insults is possible, however result showing that older SAM admissions have worse outcomes confirm that <2 years is the optimal window for reversing insults. Catch-up of linear height is generally thought to be a good thing. However, we know that when undernutrition during development is followed by improved nutrition, many animals and plants conduct accelerated or compensatory growth, which has costs. One theory is that a higher rate of cell division causes more rapid shortening of telomeres which hastens cell death and organ degradation[315]. It is difficult to separate the costs of the insult and the costs of the compensatory growth, however some now believe that catch up growth is more detrimental than the original insult. Catch-up growth can be defined as either gain in height-for-age or gain in weight-for-height; evidence of the adverse long-term effects in weight-for-height is currently more conclusive than that for gain in height-for-age[316]. There are some studies presenting the risks of rapid gain in HAZ; LBW infants who gained substantial height by 7 years of age had higher risk of coronary heart disease in adulthood[45]. This could be due to excess metabolic demands placed on organs which are either small or have fewer but larger cells following catch-up growth[40]. Could this be a consideration for SAM survivors? Their lung function may be healthy now, but will it continue to be so if they catch-up more height? "Rapid catch-up" is usually defined as an increase >0.66 z scores[317]; these SAM survivors not only had a change in WAZ of 1.13 z scores during SAM treatment, but also a change of 1.07 WAZ since discharge. HAZ changed very little during treatment and up until 1 year post-discharge however it has increased on average 1.46 z scores by 7 years post-discharge. I explored whether time taken to recover (i.e. time taken to achieve discharge weight) affected long-term growth outcomes and found no association. Despite the generally negative implication of catch-up growth, a study in Guatemala found that acquisition of length in first 3 years of life in a stunted population was not associated with increases in fat mass or abdominal fat[318]. Another study found that partial reversal of stunting in stunted children resulted in better cognitive function[263]. Stunting also

has its own host of associated risks[96]. Hence, in the absence of evidence that catch-up growth from stunting has adverse long-term implication, immediate benefits of catch-up currently outweigh potential long-term effects. Some could even argue that catch-up of linear growth will not mitigate the effects of DOHaD as the damage has already been done by the nutritional insult. These results have only given us a snap-shop during mid-childhood into some of the effects of SAM. Whether these effects, negative or negligible, will endure, diminish or grow beyond adolescence and into adulthood can only be speculated, and requires further follow-up. As with conclusions from LBW studies, the greatest policy message from these results in the importance of prevention rather than cure.

"They are as sick that surfeit with too much as they that starve with nothing."

William Shakespeare, Merchant of Venice

8.9.1 Strengths and Limitations

Strengths: Many of the strengths of this study lie within the original cohort; it is a unique cohort which as far as we know is one of the largest SAM cohorts in the region. There is also extensive baseline data on the case children during admission, discharge and at 1 year post-discharge which this study made use of. The follow-up rate in the study is excellent with relatively little loss to follow-up considering the 7 year gap since recruitment, and the difficult nature of locating participants in a setting with no addresses or communication technology. There is no evidence of bias due to loss to follow-up, the cases and community controls are well matched regarding basic demographics, data regarding most potential confounders was collected and adjusted for and the sample size for both cases and controls was more than adequately powered for all but one of the main analyses.

Limitations: There are some limitations to this study which should be mentioned. We do not have accurate data for birth weight, a key potential confounder; although we have a rough gage for case children, we do not have any information on birth weight for sibling and community controls. This information was not routinely recorded when most of these children were born and carers recollections are likely to be unreliable with children this age. We also do not have longitudinal growth data for the community control group who were only recruited at this phase of follow-up. Although sample size is very good for other outcomes, it is not sufficient to detect a significant difference for physical activity. In addition, data collectors were not blinded to the study group status of the children due to risk of data recording errors, however there were few measurements which could be affected by subjectivity. Lastly, BIA data was not calibrated with isotope dilution data, as is the gold standard, due to budget limitations, however BIVA offers a good alternative.

8.9.2 Causality

It is worth mentioning a caution regarding causality. Causality cannot be confirmed using an observational study design, however some of the criteria of causality outlined by Austin Bradford-Hill, which have been widely adopted by epidemiologists, have been met by these study results[319]. The strength of the association between SAM and the effected outcomes is robust and consistent. They are also generally consistent with hypotheses generated based on existing pre-natal and early life evidence. There is a biological gradient as the age of admission has a graded impact on outcomes. And lastly, it is biologically plausible and coherent with some current knowledge. Not all the criteria are met; severity of SAM does not exhibit a dose-dependent response on the outcomes, and the temporal sequence of the association cannot be confirmed as we do not know body composition or physical function of the cohort prior to SAM.

8.9.3 Generalisability

It is important to comment that these results are not wholly generalizable to all SAM sufferers. Firstly, survivor bias is an important consideration in this study as 46% (476/1024) of the cohort are known to have died. In cohorts where more SAM sufferers survive, results could be quite different. Survivor bias has been implicated in another study that followed-up survivors of SAM, stating that discharged SAM survivors had better WAZ and HAZ than sibling controls for this reason[91]. SAM survivors do not have significantly better results to controls in any of the outcomes studies here however survivor bias could be diminishing some of the long-term effects. Survivor bias may be most evident in the HIV positive SAM survivors who have an even higher rate of mortality and who have better HAZ and lean mass outcomes than HIV positive sibling controls.

Secondly, this cohort was started when inpatient treatment was recommended for all SAM cases, and cases were identified using NCHS reference data. The reference data and SAM treatment protocol has since changed; a more inclusive WHO reference standard has been introduced and community management of acute malnutrition (CMAM) is now widely used[320]. This could mean that SAM sufferers of today are receiving earlier treatment and will therefore experience fewer long-term effects, or conversely CMAM could be facilitating a higher survival rate which could result in more long-term adverse effects as the most affected children who would previously have died are now surviving. A long-term follow-up of a CMAM cohort is required to assess generalisability to this group.

8.10 Study Implications

8.10.1 Scientific & Programme Implications

This study has added new evidence to existing scientific knowledge; namely:

- An episode of SAM can have long-term implications
- Catch-up of linear growth post-SAM does occur, even without intervention or change of environment
- Growth patterns observed in previous DOHaD studies do seem to affect SAM survivors in the form of "brain-sparing" growth and limb-compromising growth; however the long-term effects do not exactly match those seen in LBW infants

As well as confirming some hypotheses, this study has also generated more questions requiring further research; namely:

- Does linear catch-up growth continue during puberty? Or does growth faltering occur at this stage?
- Can nutrition interventions in adolescence improve linear catch-up growth in stunted children?
- Are there increased risks of NCDs for SAM survivors who catch-up their linear height?
- Does lung function remain relatively good after puberty, even if catch up growth occurs?
- Is there any long-term effect of SAM on gut microbiome in this cohort?
- Is physical activity (measured by accelerometers) significantly lower in long-term survivors of SAM compared to controls (required sample size ~300 per group)?
- Can physical function interventions post-SAM or in adolescence improve physical strength and capacity of SAM survivors?
- How would a physical function intervention be delivered in different cultural and economic situations?

Potential Interventions: The results of this study have highlighted better prevention of SAM, reversal of stunting, increase in lean mass and improvements in physical strength and capacity as potential aims for interventions in order to ultimately improving long-term outcomes in SAM survivors. With adolescence being a time of significant developmental transition, setting the stage for adult health, this seems one optimal time for implementation of these interventions in SAM survivors[93]. A recent review highlights that interventions to promote physical activity, healthy lifestyle, improved access to nutritious and healthy foods, as well as sexual and reproductive health, have successfully improved health outcomes in young adolescents generally[321]. Delivering these interventions in schools has been evaluated and deemed effective, however more

novel methods such as use of social media and information technologies, cash transfers, social protection, and micro-finance initiatives could also be promising methods of delivering health and nutrition interventions to adolescents[321]. Future research should focus on confirming long-term effects, identifying suitable interventions to prevent or reverse long-term effects, and develop sustainable and cost-effective delivery methods for interventions.

Following future research, there could also be some changes made to SAM treatment programmes in order to mitigate some of the long-term effects observed. Ideas at this stage include active case findings and CMAM treatment for infants <6 months with MAM or SAM, and better follow-up care post-SAM, including health monitoring with health messaging and personalised advice as this population are at higher risk of mortality, nutrition supplementation and physical activity interventions, particularly for older admissions who have worse outcomes.

8.10.2 Policy Implications

Although policy is not purely developed on scientific evidence, results of scientific studies should play a role in developing future policy[322]. The results of this study have implications for future policies across the areas of child nutrition, adult health particularly NCDs, and wider nutrition-specific and development areas such as agriculture and sanitation. The over-arching policy message that can be gleaned from these results is the need for a focus not only on the mortality outcomes of SAM but the morbidity outcomes too; the emphasis shift from "surviving" to "thriving" which has gathered pace in other areas of child health need also be applied to SAM. Although the SDGs have highlighted the importance of tackling SAM, the lasting effects are not yet recognised. These results support the need to include SAM in policies concerning the long-term implications of malnutrition; many initiatives, such as SUN Movement (Scaling Up Nutrition), have their focus purely on the long-term effects of stunting. The role that SAM plays in the pathogenesis of stunting, as well as its own long-term implications, should be recognised.

8.11 Conclusions

Overall, my PhD research has shown that there are some long-term implications of an acute episode of SAM in the areas of growth, body composition and physical function. Deficits in HAZ, lean mass and grip strength are most notable in their potential health implications. Lung function, sitting height, head circumference and fat distribution measured by skinfold thickness appear unaffected by SAM at this age and while the SAM survivors remain thin. Catch up of linear growth has occurred since discharge, suggesting growth interventions post-"first 1000 days" can still be effective. However as children get older, an episode of SAM can have greater detrimental effects, emphasising the need to take every precaution to prevent SAM in children >2 years, as well as working to identify and treat children <2 years as early as possible. Other interventions to consider for SAM survivors may be further nutrition supplementation and/or physical fitness for building of lean mass and strength. It is important to note that adolescence is a time of significant developmental transition so it will be interesting to study the long-term effects of SAM after this time.

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Appendix 1: Information Sheet and Consent Form

You will be given a copy of this information sheet.

Title of Project: *Chronic Disease Outcomes following Severe Acute Malnutrition (ChroSAM study) – a prospective cohort study*

This study has been approved by the Ethics Committee [Project ID Number]: **P.02/13/1342**

Name, Address and Contact Details of Investigator: **Natasha Lelijveld**

c/o Malawi-Liverpool-Wellcome Trust Clinical Research Programme

Telephone 0996 283155

We would like to invite you to participate in this research project.

WHAT IS THE RESEARCH ABOUT?

Severe Malnutrition is a major problem for children in Malawi and many other similar countries. Lots of people known about the risks of death following malnutrition. However, children who survive can also experience problems with their future health. Some of these problems might even last into adult life. Possible problems include effects on growth, development, school achievement and health of the body (e.g. heart, lungs, physical fitness/strength). In this research we want to understand what the long term effects of malnutrition are. We will do this by comparing children who have been malnourished with those who have not (siblings and neighbours). We hope that the results of this research will help us and others to improve the treatment of malnutrition in the future so as to minimise any harmful long term effects. Your contribution to the research is therefore very valuable.

WHAT DOES THE RESEARCH INVOLVE: (There are two parts to this research)

PART (1) will take place at your home. We would like to ask you some questions about your child and your family. We would also like to take some weight, height and arm/head circumference measurements to see how your child is growing and how his/her health is (show blood pressure cuff and oxygen sats machine). We would also like to measure your (mother's) weight, height and blood pressure.

We are doing all this to help us understand how children are doing and what are possible reasons why some children are doing better and others doing not so well after malnutrition.

PART (2) will take place at Queen Elizabeth Hospital Malawi. The aim of this is do some more

detailed tests. We will arrange a visit on a mutually convenient day. You will be one of several children visiting for the same project and will be asked to arrive by 9am. We will ask some more questions and do some more tests (blowing into a machine to test how well his/her lungs are working; doing some more measurement of the body; checking his/her fitness). We would also like to take a blood sample which will tell us about any other health problems he/she may have. Part of this will be stored for possible future analysis. The visit will take about 2 hours – food and transport expenses will be provided.

With both parts of the assessment, we will let you know if we find any health problems and will advise you on what you can do / where you can go for treatment.

ABOUT TAKING PART IN RESEARCH

You should only participate if you want to; choosing not to take part will not disadvantage you or your child in any way. Before you decide whether you want to take part, it is important for you to understand what the project involves and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information.

It is up to you to decide whether to take part in both, or one part of this study, or not at all. You also do not have to answer any questions that you are not comfortable with. If you decide to take part you are still free to withdraw at any time and without giving a reason.

Please complete this form after you have read the Information Sheet and/or listened to an explanation about the research.

Title of Project: *Chronic Disease Outcomes following Severe Acute Malnutrition (ChroSAM study) – a prospective cohort study*

This study has been approved by the Ethics Committee [Project ID Number]: **- P.02/13/1342**

Thank you for considering to take part in this research. The person organising the research must explain the project to you before you agree to take part.

(for ex-MOYO children only) This is a follow-up to previous research that you have been involved with on MOYO ward, Queen Elizabeth Hospital.

If you have any questions arising from the Information Sheet or explanation already given to you, please ask the researcher before you decide whether to join in. You will be given a copy of this Consent Form to keep and refer to at any time.

I understand that if I decide at any other time during the research that I no longer wish to participate in this project, I can notify the researchers involved and be withdrawn from it immediately.

I consent to the processing of my personal information for the purposes of this research study. I understand that such information will be treated as strictly confidential.

Carer's Signature or thumbprint **Child / adolescent signature or thumbprint (if aged <18 and able to respond concerning participation ➔ assent to take part)**

I …….. AND I, ……..

agree that the research project named above has been explained to me to my satisfaction and I agree to take part in a) part 1 b) part 2 of the study. ***(circle appropriately)***
The notes written above and the Information Sheet about the project have been read to me and I understand what the research study involves.

Signed:
Date: :

Researcher's Signature. I ……..

confirm that I have carefully explained the purpose of the study to the participant and that consent is freely given

Signed:
Date:

Appendix 2: Letter from School explaining absence

Ms Natasha Lelijveld
Malawi Liverpool Wellcome Trust
College of Medicine
PO Box 30096
Chichiri
Blantyre 3

Dear Head Teacher,

This is a letter to inform you that the child ……
(name) will be missing 1 day of school on …… of ……. (date) as he/she is taking part in a medical research project called ChroSAM. This is related to a previous admission that the child had to Queen Elizabeth Central Hospital, Blantyre, in 2006/2007.

The research is taking place at the same Queen Elizabeth Central Hospital. It is hoped that the results of this research project will help the children of Malawi through better management of illnesses.

The ChroSAM project has been approved by College of Medicine Ethical Research Committee. Should you have any queries regarding this research project, please feel free to contact the Ethical Research Committee, quoting project number P.02/13/1342, on the following telephone numbers:

01877 245 / 01877 291

We would like to thank you very much for your understanding and thank you for supporting this project.

Kind Regards

Natasha Lelijveld

…….
Project Coordinator
0996 283155

Appendix 3: Ethical approval certificate COMREC

CERTIFICATE OF ETHICS APPROVAL

This is to certify that the College of Medicine Research and Ethics Committee (COMREC) has reviewed and approved a study entitled:

P.02/13/1342 - Chronic Disease Outcomes following Severe Acute malnutrition (ChroSAm study)- a prospective cohort by Dr. M. Kerac

On 22 October 2014

As you proceed with the implementation of your study, we would like you to adhere to international ethical guidelines, national guidelines and all requirements by COMREC as indicated on the next page

Dr. V. Mwapasa, Chairperson (COMREC)

Approved by
College of Medicine
22 OCT 2014
(COMREC)
Research and Ethics Committee

Date 22/10/14

Appendix 4: Ethical approval letter UCL REC

UCL RESEARCH ETHICS COMMITTEE
GRADUATE SCHOOL OFFICE

Dr Andrew Seal
Institute for Global Health
UCL

19 June 2013

Dear Dr Seal

Notification of Ethical Approval
Project ID: 4683/001: Chronic disease outcomes following severe acute malnutrition (ChroSAM study) – a prospective cohort study

I am pleased to confirm that your study has been approved by the UCL Research Ethics Committee for the duration of the project i.e. **until June 2016.**

Approval is subject to the following conditions:

1. You must seek Chair's approval for proposed amendments to the research for which this approval has been given. Ethical approval is specific to this project and must not be treated as applicable to research of a similar nature. Each research project is reviewed separately and if there are significant changes to the research protocol you should seek confirmation of continued ethical approval by completing the 'Amendment Approval Request Form'.

The form identified above can be accessed by logging on to the ethics website homepage: http://www.grad.ucl.ac.uk/ethics/ and clicking on the button marked 'Key Responsibilities of the Researcher Following Approval'.

2. It is your responsibility to report to the Committee any unanticipated problems or adverse events involving risks to participants or others. Both non-serious and serious adverse events must be reported.

   Reporting Non-Serious Adverse Events
   For non-serious adverse events you will need to inform Helen Dougal, Ethics Committee Administrator (ethics@ucl.ac.uk), within ten days of an adverse incident occurring and provide a full written report that should include any amendments to the participant information sheet and study protocol. The Chair or Vice-Chair of the Ethics Committee will confirm that the incident is non-serious and report to the Committee at the next meeting. The final view of the Committee will be communicated to you.

   Reporting Serious Adverse Events
   The Ethics Committee should be notified of all serious adverse events via the Ethics Committee Administrator immediately the incident occurs. Where the adverse incident is unexpected and serious, the Chair or Vice-Chair will decide whether the study should be terminated pending the opinion of an independent expert. The adverse event will be considered at the next Committee meeting and a decision will be made on the need to change the information leaflet and/or study protocol.

Appendix 5: Gantt Chant of study timing

	2013			2014				2015				2016
	Q2	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**
Planning												
Ethical Approval												
UK-based training (anthropometry and spirometry)												
Literature Review												
Questionnaire development												
Recruiting study nurses and field workers												
Team training & piloting												
Data												
Recruitment & Data Collection												
Quality Control												
Data Analysis												
Dissemination												
Writing up thesis												
Dissemination of results (conferences)												
Dissemination of results (journal articles)												

Appendix 6: Paper versions of data collection forms (forms B, C, H, I, and J)

Appendix 7: Questionnaires (forms A, D, E and K) (English versions)

C1 PID — Participant ID [ ][ ][ ][ ] - [ ]

A –moyo child
B –si**B**ling control
C –**C**ommunity conrol

C2 PIN — Participant Initials [ ][ ]

C3 INTDT — Interview Date [ ][ ] / [ ][ ][ ] / [2][0][ ][ ]
D D M O N Y Y Y Y

Mother Anthropometry
(must be biological mother)

C4 MDOB — Mother's Date of Birth [ ][ ][ ] / [ ][ ][ ][ ]

Enter 999 if don't know

C4 MLIV — Is Mother alive? ☐ Yes ☐ No ☐ Don't know

C4 MDOD — Mothers Date of Death (if died) [ ][ ][ ] / [ ][ ][ ][ ]

C4 MWGT — **Weight** [ ][ ][ ].[ ][ ]

If anthro not done, use following codes in place of 'Weight':

*Dead = 666.6
*Alive but not available = 555.5
*Alive but out = 444.4
(coming back)

C5 MMU — **MUAC**

Operator 1 Initials [ ][ ] [ ][ ][ ] (1 2 3)

Operator 2 Initals [ ][ ] [ ][ ][ ] (1 2 3)

C5 MMU_FIN Combined MUAC (mm) [ ][ ][ ].[ ]

C6 MHT — **Height (standing)**

Operator 1 Initials [ ][ ] [ ][ ][ ] (1 2 3)

Operator 2 Initals [ ][ ] [ ][ ][ ] (1 2 3)

C6 MHT_FIN Combined Height (cm) [ ][ ][ ].[ ]

C7 MSHT — **Sitting Height**

Operator 1 Initials [ ][ ] [ ][ ][ ] (1 2 3)

Operator 2 Initals [ ][ ] [ ][ ][ ] (1 2 3)

C7 MSHT_FIN Combined Sitting Height (cm) [ ][ ][ ].[ ]

C8 PID Participant ID ☐☐☐☐ - ☐ A/B/C

C9 PIN Participant Initials ☐☐

Father Anthropometry
(Must be biological Father)

C10 FDOB Father's Date of Birth ☐☐☐ / ☐☐☐☐ Enter 999 if don't know

C10 FLIV Is Father alive? ☐ Yes ☐ No ☐ Don't know

C10 FDOD Father's Date of Death (if died) ☐☐☐ / ☐☐☐☐

C10 FWGT **Weight** ☐☐☐.☐☐

*Dead = 666.6
*Alive but not available = 555.5
*Alive but out = 444.4
(coming back)

C11 FMU **MUAC**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C11 FMU_FIN Combined MUAC (mm) ☐☐☐.☐

C12 FHT **Height (standing)**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C12 FHT_FIN Combined Height (cm) ☐☐☐.☐

C13 FSHT **Sitting Height**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C13 FSHT_FIN Combined Sitting Height (cm) ☐☐☐.☐

C14 PID Participant ID ☐☐☐☐ - ☐ A/B/C

C15 PIN Participant Initials ☐☐

C16 NKIDS How many children are there in the family? (alive) (children must all have the same mother) ☐☐

1st Born Anthropometry
(Unless Moyo/Control Child)

C16 1DOB Month and Year of Birth ☐☐☐ / ☐☐☐☐ (M M M / Y Y Y Y)

If moyo child, write **MMM** in place of Month of birth and do not complete. If Sib control write **SSS**. If community control write **CCC**

C17 1DOD If died, year of death ☐☐☐☐ (Y Y Y Y)

C18 1SEX **Sex** ☐ Male ☐ Female

Don't need to measure if child is less than 2 years.

C19 1WGT **Weight** ☐☐☐.☐☐

*Died = 666.6
*Not Applicable = 777.7
*Alive but not available = 555.5
*Alive but out = 444.4 (coming back)
*Less than 2 years = 222.2

C20 1MU **MUAC**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C20 1MU_FIN Combined MUAC (mm) ☐☐☐

C21 1HT **Height (standing)**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C21 1HT_FIN Combined Height (cm) ☐☐☐.☐

C23 PID Participant ID ☐☐☐☐ - ☐ A/B/C

C24 PIN Participant Initials ☐☐

2nd Born Anthropometry
(Unless Moyo or Control child)

C25 2DOB Month and Year of Birth ☐☐☐ / ☐☐☐☐ (M M M / Y Y Y Y)

If moyo child, write **MMM** in place of Month of birth and do not complete. If Sib control write **SSS.** If community control write **CCC**

C26 2DOD If died, year of death ☐☐☐☐ (Y Y Y Y)

C27 2SEX **Sex** ☐ Male ☐ Female

C28 2WGT **Weight** ☐☐☐.☐☐

*Died = 666.6
*Not Applicable = 777.7 (ie no second born)
*Alive but not available = 555.5
*Alive but out = 444.4 (coming back)
*Less than 2 years = 222.2

C29 2MU **MUAC**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C29 2MU_FIN Combined MUAC (mm) ☐☐☐

C30 2HT **Height (standing)**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C30 2HT_FIN Combined Height (cm) ☐☐☐.☐

C32 PID Participant ID ☐☐☐☐ - ☐ A/B/C

C33 PIN Participant Initials ☐☐

3rd Born Anthropometry
(if not Moyo or Control child)

C34 3DOB Month and Year of Birth ☐☐☐ / ☐☐☐☐ (M M M / Y Y Y Y)

If moyo child, write MMM in place of Month of birth and do not complete. If Sib control write SSS. If community control write CCC)

C35 3DOD If died, year of death ☐☐☐☐ (Y Y Y Y)

C36 3SEX **Sex** ☐ Male ☐ Female

C37 3WGT **Weight** ☐☐☐.☐☐

*Died = 666.6
*Not Applicable = 777.7 (ie. no third born)
*Alive but not available = 555.5
*Alive but out = 444.4 (coming back)
*Less than 2 years = 222.2

C38 3MU **MUAC**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C38 3MU_FIN Combined MUAC (mm) ☐☐☐

C39 3HT **Height (standing)**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C39 3HT_FIN Combined Height (cm) ☐☐☐.☐

C41 PID Participant ID [ ][ ][ ][ ] - [ ] A/B/C

C42 PIN Participant Initials [ ][ ]

4th Born Anthropometry
(unless Moyo or Control child

C43 4DOB Month and Year of Birth [ ][ ][ ] / [ ][ ][ ][ ]
M M M Y Y Y Y

If moyo child, write MMM in place of Month of birth and do not complete. If Sib control write SSS. If community control write CCC)

C44 4DOD If died, year of death [ ][ ][ ][ ]
Y Y Y Y

C45 4SEX **Sex** ☐ Male ☐ Female

C46 4WGT **Weight** [ ][ ][ ].[ ][ ]

*Died = 666.6
*Not Applicable = 777.7 (ie no 4TH born)
*Alive but not available = 555.5
*Alive but out = 444.4 (coming back)
*Less than 2 years = 222.2

C47 4MU **MUAC**

Operator 1 Initials [ ][ ] [ ][ ][ ] (1 2 3)

Operator 2 Initals [ ][ ] [ ][ ][ ] (1 2 3)

C47 4MU_FIN Combined MUAC (mm) [ ][ ][ ]

C48 4HT **Height (standing)**

Operator 1 Initials [ ][ ] [ ][ ][ ] (1 2 3)

Operator 2 Initals [ ][ ] [ ][ ][ ] (1 2 3)

C48 4HT_FIN Combined Height (cm) [ ][ ][ ].[ ]

C50 PID Participant ID ☐☐☐☐ - ☐ A/B/C

C51 PIN Participant Initials ☐☐

5th Born Anthropometry
(unless Moyo or Control child

C52 5DOB Month and Year of Birth ☐☐☐ / ☐☐☐☐ (M M M / Y Y Y Y)

If moyo child, write **MMM** in place of Month of birth and do not complete. If Sib control write **SSS.** IF community control write **CCC**

C53 5DOD If died, year of death ☐☐☐☐ (Y Y Y Y)

C54 5SEX **Sex** ☐ Male ☐ Female

C55 5WGT **Weight** ☐☐☐.☐☐

*Died = 666.6
*Not Applicable = 777.7 (ie no 5TH born)
*Alive but not available = 555.5
*Alive but out = 444.4 (coming back)
*Less than 2 years = 222.2

C56 5MU **MUAC**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C56 5MU_FIN Combined MUAC (mm) ☐☐☐

C57 5HT **Height (standing)**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C57 5HT_FIN Combined Height (cm) ☐☐☐.☐

C59 PID Participant ID ☐☐☐☐ - ☐ A/B/C

C60 PIN Participant Initials ☐☐

6th Born Anthropometry
(Unless Moyo/Control Child)

C61 6DOB Month and Year of Birth ☐☐☐ / ☐☐☐☐ (M M M / Y Y Y Y)

If moyo child, write MMM in place of Month of birth and do not complete. If Sib control write SSS. IF community control write CCC)

C62 6DOD If died, year of death ☐☐☐☐ (Y Y Y Y)

C63 6SEX **Sex** ☐ Male ☐ Female

C64 6WGT **Weight** ☐☐☐.☐☐

*Died = 666.6
*Not Applicable = 777.7 (ie no 6TH born)
*Alive but not available = 555.5
*Alive but out = 444.4 (coming back)
*Less than 2 years = 222.2

C65 6MU **MUAC**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C65 6MU_FIN Combined MUAC (mm) ☐☐☐

C66 6HT **Height (standing)**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C66 6HT_FIN Combined Height (cm) ☐☐☐.☐

C68 PID Participant ID ☐☐☐☐ - ☐ A/B/C

C69 PIN Participant Initials ☐☐

7th Born Anthropometry
(Unless Moyo/Control Child)

C70 7DOB Month and Year of Birth ☐☐☐ / ☐☐☐☐ (M M M / Y Y Y Y)

If moyo child, write MMM in place of Month of birth and do not complete. If Sib control write SSS. IF community control write CCC)

C71 7DOD If died, year of death ☐☐☐☐ (Y Y Y Y)

C72 7SEX **Sex** ☐ Male ☐ Female

C73 7WGT **Weight** ☐☐☐.☐☐

*Died = 666.6
*Not Applicable = 777.7 (ie no 7TH born)
*Alive but not available = 555.5
*Alive but out = 444.4 (coming back)
*Less than 2 years = 222.2

C74 7MU **MUAC**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C74 7MU_FIN Combined MUAC (mm) ☐☐☐

C75 7HT **Height (standing)**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C75 7HT_FIN Combined Height (cm) ☐☐☐.☐

C77 PID Participant ID ☐☐☐☐ - ☐ A/B/C

C78 PIN Participant Initials ☐☐

8th Born Anthropometry
(Unless Moyo/Control Child)

C79 8DOB Month and Year of Birth ☐☐☐ / ☐☐☐☐ (M M M / Y Y Y Y)

If moyo child, write MMM in place of Month of birth and do not complete. If Sib control write SSS. IF community control write CCC)

C80 8DOD If died, year of death ☐☐☐☐ (Y Y Y Y)

C81 8SEX **Sex** ☐ Male ☐ Female

C82 8WGT **Weight** ☐☐☐.☐☐

*Died = 666.6
*Not Applicable = 777.7 (ie no 8TH born)
*Alive but not available = 555.5
*Alive but out = 444.4 (coming back)
*Less than 2 years = 222.2

C83 8MU **MUAC**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C83 8MU_FIN Combined MUAC (mm) ☐☐☐

C84 8HT **Height (standing)**

Operator 1 Initials ☐☐ ☐☐☐ (1 2 3)

Operator 2 Initals ☐☐ ☐☐☐ (1 2 3)

C84 8HT_FIN Combined Height (cm) ☐☐☐.☐

H1	PID	Participant ID ☐☐☐☐ - ☐ A/B/C	Participant ID = HMIS number followed by A –moyo child B –si**B**ling control C –**C**ommunity onrol
H2	PIN	Participant Initials ☐☐	
H3	OPIN	Operator Initials ☐☐	
H4	INTDT	Interview Date ☐☐ / ☐☐☐ / ☐☐ D D M M M Y Y	
H5	MBP	Mother's Blood pressure ☐☐☐ / ☐☐	
H6	BP	Blood pressure(child) ☐☐☐ / ☐☐	
H7	SpO2	Oxygen saturation (%) ☐☐☐ %	

Hand Grip Strength

H8 LHRH Is the child left or right-handed? ☐ Right ☐ Left

H9 GRPL Hand grip strength LEFT ☐☐.☐ (Attempt 1) ☐☐.☐ (Attempt 2) ☐☐.☐ (Attempt 3)

LBEST Best score on LEFT: ☐☐.☐

H10 GRPR Hand grip strength RIGHT ☐☐.☐ (Attempt 1) ☐☐.☐ (Attempt 2) ☐☐.☐ (Attempt 3)

RBEST Best score on RIGHT: ☐☐.☐

H11 TECH Any technical difficulties which may affect the results? ☐ Yes ☐ No

If yes, please specify on Master form Z

H12		Participant ID ☐☐☐☐ - ☐ A/B/C
H13		Participant Initials ☐☐

Bioelectrical Impedance Analysis (BIA)

H14	BIAN1	Test Number (reading 1) ☐☐
H15	BIAN2	Test Number (reading 2) ☐☐

Reading 1:

H16	BIAZ	Impedance (Z) at 50khz ☐☐☐☐
H17	BIAR	Resistance (R) at 50khz ☐☐☐☐
H18	BIAXc	Reactance (Xc) at 50khz ☐☐☐.☐
H19	BIAPA	Phase angle at 50khz ☐.☐

Reading 2:

H20	BIAZ	Impedance (Z) at 50khz ☐☐☐☐
H21	BIAR	Resistance (R) at 50khz ☐☐☐☐
H22	BIAXc	Reactance (Xc) at 50khz ☐☐☐.☐
H23	BIAPA	Phase angle at 50khz ☐.☐

Accelerometers

H24	ACEL	Accelerometer Given? ☐ Yes ☐ No
H25	ACELD	If yes, device number: ☐☐☐☐

I1	PID	Participant ID	☐☐☐☐ - ☐ A/B/C
I2	PIN	Participant Initials	☐☐

Participant ID = HMIS number followed by
A –moyo child
B –si**B**ling control
C –**C**ommunity conrol

I3	INTDT	Date of Hospital visit	☐☐ / ☐☐☐ / ☐☐ (D D / M M M / Y Y)

Skinfold thickness

I4		Left Biceps (FRONT) (mm)		
	SFBOP1	Operator 1 Initials	☐☐ ☐☐☐ (1 2 3)	I4SFB_FIN Combined Biceps Skinfold
	SFB0P2	Operator 2 Initials	☐☐ ☐☐☐ (1 2 3)	☐☐.☐
I5		Left Triceps (BACK) (mm)		
	SFTOP1	Operator 1 Initials	☐☐ ☐☐☐ (1 2 3)	I5SFT_FIN Combined Tricep Skinfold
	SFTOP2	Operator 2 Initials	☐☐ ☐☐☐ (1 2 3)	☐☐.☐
I6		L Subscapular (shoulder) (mm)		
	SFSOP1	Operator 1 Initials	☐☐ ☐☐☐ (1 2 3)	I6SFS_FIN Combined Subscapular Skinfold
	SFSOP2	Operator 2 Initials	☐☐ ☐☐☐ (1 2 3)	☐☐.☐
I7		L Suprailiac (waist)(mm)		
	SFWOP1	Operator 1 Initials	☐☐ ☐☐☐ (1 2 3)	I7SFW_FIN Combined Subscapular Skinfold
	SFWOP2	Operator 2 Initials	☐☐ ☐☐☐ (1 2 3)	☐☐.☐

I8	PID	Participant ID	☐☐☐☐ - ☐
I9	PIN	Participant Initials	☐☐
I10	OPIN	Operator Initials	☐☐

Spirometry

I11	SPIC1	Any cough/cold/flu at the moment? (according to carer)	☐ Yes ☐ No ☐ Dont know
I12	SPIC2	Any cough observed?	☐ Yes ☐ No
I13	SPIC3	Any runny nose observed?	☐ Yes ☐ No

Does mother or father have any of the following:

I14	FAMAS	Asthma?	☐ Yes ☐ No ☐ Dont know
I15	FAMWH	Wheeze?	☐ Yes ☐ No ☐ Dont know
I16	FAMAL	Allergies?	☐ Yes ☐ No ☐ Dont know

Does child have any of the following contraindications? (ask carer)

I17	SPIC4	Chest pain?	☐ Yes ☐ No
I18	SPIC5	Heart problems?	☐ Yes ☐ No
I19	SPIC6	Surgery or hospital admission in past month?	☐ Yes ☐ No
I20	SPIC7	Current chest infection?	☐ Yes ☐ No
I21	SPIC8	Coughing blood?	☐ Yes ☐ No

If the answer is yes to any of the above (I17-I20), do NOT perform spirometry. Report to a clinician (ie. Dr Kerac or Dr Vostij).

I22	SPI	Spirometry test done?	☐ Yes ☐ No

Add any additional notes to Master form Z

Tick Form Z Checklist when Bloods, Retina photos, MRI scan, Ultrasound and Salivery Cortisol done.

J1	PID	Participant ID	☐☐☐☐ - ☐ A/B/C
J2	PIN	Participant Initials	☐☐
J3	OPIN	Operator Initials	☐☐
J4	STPC1	Any technical difficulty which would inhibit test?	☐ Yes ☐ No
		If yes, specify on Master form Z	
		Does child have any of the following contraindications? (ask carer)	
J5	STPC2	Heart problems?	☐ Yes ☐ No
J6	STPC3	Lung problems?	☐ Yes ☐ No
J7	STPC4	Asthma but no inhaler?	☐ Yes ☐ No
J8	STPC5	Mental or physical impairment which prevents cooperation?	☐ Yes ☐ No
		If the answer is yes to any of the above, do not perfrom I step test	
J9	STPH	Step height	☐ 15CM ☐ 20CM ☐ 25CM

		LEVEL	TIME	SpO2 %	Heart Rate (BPM)	OMNI (0-10)	VAS (0-10)
J10B		Base Line	00	☐☐☐	☐☐☐	☐☐	☐☐
J10		Level 1	1 min	☐☐☐	☐☐☐		
J11			2 min	☐☐☐	☐☐☐	☐☐	
J12		Level 2	3 min	☐☐☐	☐☐☐		
J13			4 min	☐☐☐	☐☐☐	☐☐	
J14		Level 3	5 min	☐☐☐	☐☐☐		
J15			6 min	☐☐☐	☐☐☐	☐☐	
J16		Level 4	7 min	☐☐☐	☐☐☐		
J17			8 min	☐☐☐	☐☐☐	☐☐	

Form J. I-Step Test. ChroSAM study.

J18	PID	Participant ID ☐☐☐☐ - ☐
J19	PIN	Participant Initials ☐☐

		LEVEL	TIME	SpO2 %	Heart Rate (BPM)	OMNI (0-10)	VAS (0-10)
J20		Level 5	9 min	☐☐☐	☐☐☐		
J21			10 min	☐☐☐	☐☐☐	☐☐	☐☐
J22		1 Min Recovery	1 Min Recovery	☐☐☐	☐☐☐	☐☐	☐☐

J23	SCOMP	Test completed?	☐ Yes ☐ No
J24	SLEVE	Number of completed levels	☐
J25	STIME	Total time completed	☐☐.☐☐ MIN.SECS
J26	SSTOP	Reasons for stopping the test	☐ Inability to maintain rhythm ☐ Sudden pallor ☐ Drop in SpO2 >10% from resting ☐ Too breathless ☐ Excessive coughing ☐ Loss of coordination ☐ Declined to continue ☐ Light headedness ☐ Not Applicable
J27	SOXL	**Oxygen Saturation: (%)**	Lowest recording ☐☐
J28	SHRH	**Heart Rate: (BPM)**	Highest recording ☐☐☐
J29	SOMH	**OMNI scale (0-10):**	Highest recording ☐☐
J30	SVAH	**VAS (0-10):**	Highest recording ☐☐

Form A. Basic Information. ChroSAM study.

A1 PID — Participant ID ☐☐☐☐ - ☐
A –moyo child
B –si**B**ling control
C –**C**ommunity conrol

A2 PIN — Participant Initials ☐☐

A3 OPIN — Operator Initials ☐☐

A4 INTDT — Date of visit ☐☐ / ☐☐☐ / ☐☐☐☐
D D / M O N / Y Y Y Y

A5 AGE — Child's reported age ☐☐ years

A6 DOB — Date of Birth ☐☐☐ / ☐☐☐☐
M O N / Y Y Y Y

Check that age and year of birth agree.

A7 SEX — Sex ☐ Male ☐ Female

A8 BRTH — Birth Order

☐ 1st (ie. oldest)
☐ 2nd
☐ 3rd
☐ 4th
☐ 5th
☐ 6th
☐ 7th
☐ 8th
☐ Other

A9 OUTC — Outcome of visit

☐ Child seen (1)
☐ Child not seen: but info reliable (2)
☐ Child not seen: info may be unreliable (3)
☐ Unsuccessful search (4)

Information may be unreliable when given by a friend or neighbour rather than by a close family member.

A10 CARQ — Which carer is answering these questions?

☐ Mother (1)
☐ Father (2)
☐ Grandmother (3)
☐ Aunt (4)
☐ Uncle (5)
☐ Neighbour (6)
☐ Other family (7)
☐ Other non-family (8)
☐ Dont know (99)

A11	PID	Participant ID	☐☐☐☐ - ☐ A/B/C
A12	PIN	Participant Initials	☐☐
A13	CARM	Who is the main carer?	☐ Mother (1) ☐ Father (2) ☐ Grandmother (3) ☐ Aunt (4) ☐ Uncle (5) ☐ Neighbour (6) ☐ Other family (7) ☐ Other non-family (8) ☐ Dont know (99)
A14	WELL	How is the child now (or when last seen)?	☐ Child died (1) (See A15 and A16) ☐ Child well (2) (proceed to A17) ☐ Child sick (3) (proceed to A17) ☐ Don't know (99) (proceed to A17)
A15	POD	If died, where did they die?	☐ Home (1) ☐ Hospital MOYO (2) ☐ Hospital QECH/PSCW (3) ☐ Hospital other (4) ☐ Dont know (99)
		Include **CAUSE OF DEATH** and any other notes on Master Checklist Z	
A16	DOD	Date of final outcome (ie. Date of death or date last known to be alive)	☐☐ / ☐☐☐ / ☐☐☐☐ (D D / M M M / Y Y Y Y) *If day or month unknown, enter 9 in its place*
		If child has died, proceed to Form E, F and G If child is alive, continue below:	
A17	PUBE	Has child started puberty? (ie. pubic hair/periods)	☐ Yes ☐ No ☐ Dont know
A18	RESAM	Any episodes of SAM.. since we first met you at Moyo / ever(controls)?	☐ Yes (see questions A19 to A27) ☐ No (proceed to A28) ☐ Dont know (proceed to A28)
A19	KWAS	Any episodes of Kwashiorkor/ swelling?	☐ Yes ☐ No
A19	KWAS1	If yes, when?	☐☐☐☐ , ☐☐☐☐ , ☐☐☐☐ (Y Y Y Y , Y Y Y Y , Y Y Y Y)

If more that 3 times, write how many times on Form Z

A20	PID	Participant ID	☐☐☐☐ - ☐ A/B/C
A21	PIN	Participant Initials	☐☐
A22	WAST	Any episodes of wasting / getting thin?	☐ Yes ☐ No
A22	WAST1	If yes, when?	☐☐☐☐ , ☐☐☐☐ , ☐☐☐☐ (Y Y Y Y)
		If more than 3 times, specify how many times on form z	
A23	MEDSAM	Did child get medical attention?	☐ Yes ☐ No ☐ Dont know
A24	NRUSAM	How many times admitted to NRU (inpatient)?	☐☐ 99 = dont know
A25	CTCSAM	How many times had RUTF/OTP only (CTC)?	☐☐ 99 = dont know
A26	ADVSAM	How many times given advice, multivits/ other, without RUTF?	☐☐ 99 = dont know
A27	SFPSAM	How many times referred to SFP alone?	☐☐ 99 = dont know
A28	INPAT	Number of inpatient admissions ever (not SAM)	☐☐ If zero, skip A32 and A33
A29	INPAT1	Number of inpatient admissions in the last 1 year (not SAM)	☐☐
A30	OUTPA	Number of outpatient visits in last 6 months (not SAM)(exclude routine visits such as ART, OTP)	☐☐ e.g. Health centre or clinic)
A31	PNEU	Ever admitted with pneumonia/ chest infection?	☐ Yes ☐ No ☐ Dont know
A32	PNAGE	If yes, at what age?	☐☐ , ☐☐ (Y Y M M) Fill in with 99, if unknown
A33	ASMA	Has child ever been diagnosed with asthma?	☐ Yes ☐ No ☐ Dont know

A34	PID	Participant ID	☐☐☐☐ - ☐ A/B/C
A35	PIN	Participant Initials	☐☐

A36	TB	Has the child ever had TB?	☐ Yes ☐ No ☐ Dont know	If Yes – see question A37–A40 If No/dont know – proceed to A41
A37	TBYR	If yes, year diagnosed with TB?	☐☐☐☐ Y Y Y Y	
A38	TBFAM	Were other children in the family screened?	☐ Yes ☐ No ☐ Dont know	
A39	TBTRT	How many months did child have treatment for?	☐☐	If not treated, enter 00
A40	TB6M	If treatment was less than 6 months, why?	☐ Still taking treatment ☐ Other (specify on Form Z) ☐ Dont know	
A41	HLTH	Has child ever been diagnosed with any of the following? (tick all that apply)	☐ Eczema or skin allergies (1) ☐ Wheeze (2) ☐ Persistent Cough (3) ☐ Other health problems (4) (Specify on Form Z) ☐ None (5)	
A42	HIVT	Has child ever had a HIV test?	☐ Yes ☐ No ☐ Dont know	If no/dont know, skip to A43
A43	HIV	What is the child's HIV status	☐ R (Positive) (1) ☐ NR (Negative) (2) ☐ Dont know (99) ☐ Prefer not to say (88)	
A44	HIVRT	Is a (re)test needed?	☐ Yes ☐ No	
A45	HIVRT1	If yes, what was done?	☐ Retest done, R (1) ☐ Retest done, NR (2) ☐ Counselled and referred for test (3) ☐ Refused test (4)	

Yes if test was done before 18months of age, or if never tested

A46	PID	Participant ID	☐☐☐☐ - ☐ A/B/C
A47	PIN	Participant Initials	☐☐

Only ask questions A48 – A60 if child is HIV reactive.
If non-reactive or never tested, skip to question A61.

A48	COT	Has child ever had COTRIMOXAZOLE PROPHYLAXIS? (1 daily dose)	☐ Yes ☐ No ☐ Dont know	If No/ Dont know, skip to A52
A49	COTDS	If yes, when did child have last dose?	☐ Today ☐ Yesterday ☐ 2 days ago ☐ 3-7 days ago ☐ 1 week -1 month ago ☐ More than 1 month ago ☐ Never since MOYO ☐ Dont know	
A50	COTWH	If yes, where got CoT from (since MOYO)?	☐ CoT clinic QECH ☐ Health centre ☐ Other ☐ Dont know	
A51	COTPR	Any problems taking daily CoT? (see codes)	☐☐ ☐☐ ☐☐	
A52	ARV	Has child ever had ARVs? (2 daily doses)	☐ Yes (1) (see A53 – A60) ☐ No, never (2) (proceed to A61) ☐ No, not eligible (3) (proceed to A61) ☐ Dont know (99) (proceed to A61)	
A53	ARVDT	Date started ARVs	☐☐ / ☐☐☐ / ☐☐☐☐ D D M O N Y Y Y Y	If day/month unknow, fill with 99

A54	PID	Participant ID	☐☐☐☐ - ☐ A/B/C
A55	PIN	Participant Initials	☐☐

A56	ARVST	Still taking ARVs?	☐ Yes ☐ No ☐ Dont know	If No/ Dont know, proceed to A60
A57	ARVDS	When did child last take their medication?	☐ Today (1) ☐ Yesterday (2) ☐ 2 days ago (3) ☐ 3-7 days ago (4) ☐ 1 week -1 month ago (5) ☐ More than 1 month ago (6) ☐ Never since MOYO (7) ☐ Dont know (99)	
A58	ARVMS	When did child last miss a dose?	☐ Missed today or within last week (1) ☐ Missed dose 1-2 weeks ago (2) ☐ Missed dose 2-4 weeks ago (3) ☐ Missed dose 1-3 months ago(4) ☐ Missed nothing last 3-12 months (5) ☐ Dont know (99)	
A59	ARVWH	Where got ARVs from?	☐ QECH (1) ☐ Health centre (2) ☐ Other (3) (Specify on Form Z) ☐ Dont know (99)	
A60	ARVPR	Were there any problems taking ARVs? (see codes)	☐☐ ☐☐ ☐☐	

End of HIV questions

A61 PID Participant ID ☐☐☐☐ - ☐ A/B/C

A62 PIN Participant Initials ☐☐

A63 SCHA What is highest level of school achievement for this child? ☐☐

A64 SCHB Did this child attend school at any time during this past year? ☐ Yes ☐ No ☐ Dont know

00= None	Primary:		Secondary:	
	01= S1	07= S7	09= F1	99= Don't Know
	02= S2	08= S8	10= F2	
	03= S3		11= F3	
	04= S4		12= F4	
	05= S5		13= F5	
	06= S6		14= F6	

A65 MEDAC Medical action following visit?

☐ No medical intervention required (1)

☐ Medical advice given (2)

☐ Medical action recommended (3) (specify on Z)

A66 STAC Study action following visit?

☐ Agreed to attend hospital 'base' appointment (1)

☐ Declined to attend 'base' appointment (2)

☐ Arranged to visit the home on a different date (3)

D1	PID	Patient ID	☐☐☐☐ - ☐ A/B/C
D2	PIN	Patient Initials	☐☐
D3	OPIN	Operator Initials	☐☐
D4	INTDT	Interview Date	☐☐ / ☐☐☐ / 2 0 ☐☐ (D D / M O N / Y Y Y Y)

Participant ID = HMIS number followed by
A –moyo child
B –si**B**ling control
C –**C**ommunity onrol

D5 PTGT — Are the biological parents still married/together?
☐ Yes ☐ No ☐ Don't know ☐ Not Applicable

D6 MOCC — What is main occupation of main female carer (use codes) ☐☐

D7 FOCC — What is main occupation of main male carer? (use codes) ☐☐

Codes:

01= mlimi/ganyu	06= worked before,but now seeking work	09= never worked, not looking
02= employee	07= worked before, not looking for work	10= housewife
03= family business	08= never worked before, but looking	11= student
04= self employed		12= other
05= employer		99= dont know

D8 MLIT — Can mother/female carer read? (use test sentance below)

The child is reading a book

Farming is hard work

☐ Yes, can read whole sentence (1)
☐ No, can't read (2)
☐ Can read only part of sentence (3)
☐ Reportedly can read, but not present (4)
☐ Don't know (99)

D9 FLIT — Can father/male carer read? (use test sentance below)

The child is reading a book

Farming is hard work

☐ Yes, can read whole sentence (1)
☐ No, can't read (2)
☐ Can read only part of sentence (3)
☐ Reportedly can read, but not present (4)
☐ Don't know (99)

PID Patient ID ☐☐☐☐ - ☐ A/B/C

PIN Patient Initials ☐☐

D10 MED Highest level of education acheived by mother/female carer? (use codes) ☐☐

D11 FED Highest level of education acheived by father/ male carer? (use codes) ☐☐

0= None	Primary:	Secondary:	15= University/other higher
	01= S1 07= S7	09= F1	16= Technical College
	02= S2 08= S8	10= F2	
	03= S3	11= F3	
	04= S4	12= F4	99= Don't Know
	05= S5	13= F5	
	06= S6	14= F6	

Please ensure PRIVACY before asking the following question:
(If privacy is not possible, skip question and move to next form)

Tell the mother: "I am now going to ask you some questions about other important aspects of a womans life. You may find some of these questions very personal, however, let me assure you that your answers will be completely confidential and will not be told to anyone else"

D12 VIOL From the time you were 15, has any one hit you, slapped you, kicked you, or done anything else to hurt you physically?

- ☐ Yes (1)
- ☐ No (2)
- ☐ No answer / won't say (88)

D13 VWHO If yes, who has hurt you in this way?

- ☐ Sister/brother (1)
- ☐ Daughter/Son (2)
- ☐ Mother/ Step-Mother(3)
- ☐ Father / Step Father (4)
- ☐ Teacher (5)
- ☐ Mother in Law (6)
- ☐ Father in Law (7)
- ☐ Current husband /partner (8)
- ☐ Previous husband/partner (9)
- ☐ Police/Soldier (10)
- ☐ Employer/Worker (11)
- ☐ Other (12) (Specify on Z)

PID Patient ID ☐☐☐☐ - ☐ A/B/C

PIN Patient Initials ☐☐

D14 SICK — Are there any members of the household who have been sick for at least 3 months in the past 12 months? (ie. too sick to work or do normal activities) ☐ Yes ☐ No ☐ Don't Know — If No/Don't know, skip to question D22 (next page)

D15 MEDSP — During the last 12 months, has your household recieved any medical support for the sick person? Such as medical care or medicine which you did not have to pay for. ☐ Yes ☐ No ☐ Don't Know

D16 EMOSP — During the past 12 months, has your household recieved any emotional or psychological support for the sick person? Such as companionship, counseling from a trained counselor, or spiritual support, which you didnt have to pay for. ☐ Yes ☐ No ☐ Don't Know

D17 MATSP — During the past 12 months, has your household recieved any material support for the sick person? Such as clothing, food or financial support, which you didnt have to pay for. ☐ Yes ☐ No ☐ Don't Know

D18 SOCSP — During the past 12 months, has your household recieved any social support for the sick person? Such as help with housework, training for a caregiver, or legal services, which you didnt have to pay for. ☐ Yes ☐ No ☐ Don't Know

D19 SICAG — What age is the sick person? ☐☐ If older than 18, skip to question D22 (next page)

D20 SCHSP — In the last 12 months, did your household receive any support for the sick child's schooling? Suck as allowance, free admission, books or supplies, which you didnt have to pay for. ☐ Yes ☐ No ☐ Don't Know

Specify type of school support below, if applicable:

D21

PID Patient ID ☐☐☐☐ - ☐ A/B/C

PIN Patient Initials ☐☐

I am now going to ask you some questions about the support you get from you friends and family:

D22 MSS1 Kodi pali munthu wofunikila m'moyo mwanu amene mungathe kugawana naye nkhawa zanu ndi chisangalalo chanu? ☐ Yes ☐ No

D23 MSS2 If 'yes', who is that person?
- ☐ Amuna anu (husband) (1)
- ☐ Wachibale wina (family member) (2)
- ☐ Mzanu (friend) (3)
- ☐ Zina (other) (specify on z) (4)

D24 MSS3 Kodi mumapeza mtendere wa mumtima kuchokera ku abale anu? ☐ Yes ☐ No

D25 MSS4 Kodi mukhonza kudalira anzanu zinthu zikavuta? ☐ Yes ☐ No

---

D26 BLKT Does (participant child) have a blanket? ☐ Yes ☐ No ☐ Don't Know

D27 SHOE Does (participant child) have a pair of shoes? ☐ Yes ☐ No ☐ Don't Know

D28 CLOTH Does (participant child) have atleast 2 sets of clothes? ☐ Yes ☐ No ☐ Don't Know

D29 MEAL How many meals did (participant child) eat yesterday? ☐

D30 SCHME Does (participant child) receive school meals? ☐ Yes ☐ No ☐ Don't Know

Participant ID = HMIS number followed by
A –moyo child
B –si**B**ling control
C –**C**ommunity conrol

E1	PID	Patient ID	☐☐☐☐ - ☐ A/B/C
E2	PIN	Patient Initials	☐☐
E3	OPIN	Operator Initials	☐☐

These questions are regarding the home of the main carer / the place where the child usually stays:

E4	RENT	Is the house...	☐ Owned ☐ Rented ☐ Other ☐ Dont know
E5	ROOM	How many sleeping rooms are there? (excl. bathroom/storerooms/garage)	☐☐
E6	MEMB	How many people usually sleep in the house together?	☐☐

In the dwelling is there:

E7	ELEC	Electricity	☐ Yes ☐ No ☐ Dont know
E8	RADIO	A radio	☐ Yes ☐ No ☐ Dont know
E9	BIKE	A bicycle	☐ Yes ☐ No ☐ Dont know
E10	MBIKE	A motorbike	☐ Yes ☐ No ☐ Dont know
E11	CAR	A car or truck	☐ Yes ☐ No ☐ Dont know
E11a	KOLO	A Koloboyi	☐ Yes ☐ No ☐ Dont know
E12	LAMP	A paraffin lamp (other than Koloboyi)	☐ Yes ☐ No ☐ Dont know
E13	OXCA	An oxcart	☐ Yes ☐ No ☐ Dont know
E14	TV	A television	☐ Yes ☐ No ☐ Dont know
E15	CELL	A cellphone	☐ Yes ☐ No ☐ Dont know
E16	LPHON	A telephone (landline)	☐ Yes ☐ No ☐ Dont know
E17	BED	A bed with a mattress	☐ Yes ☐ No ☐ Dont know
E18	SOFA	A sofa set	☐ Yes ☐ No ☐ Dont know
E19	TABL	A table and chair(s)	☐ Yes ☐ No ☐ Dont know
E20	FRID	A refridgerator	☐ Yes ☐ No ☐ Dont know
E21	WATCH	A watch	☐ Yes ☐ No ☐ Dont know
E21a	MOTO	A motocycle	☐ Yes ☐ No ☐ Dont know

E22	PID	Patient ID	☐☐☐☐ - ☐ A/B/C
E23	PIN	Patient Initials	☐☐
E24	FARM	Do members of the household work their own agricultural land?	☐ Yes ☐ No ☐ Dont know
E25	WATE	What is the principal household source of drinking water?	☐ Pipe inside dwelling (1) ☐ Pipe outside dwelling /to yard (2) ☐ Protected borehole/well (3) ☐ Public tap (4) ☐ Traditional public well (5) ☐ River/canal/lake (6) ☐ Tanker Truck (7) ☐ Bottled water (8) ☐ Rainwater (9) ☐ Cart with small tank (10) ☐ Don't know (99)
E26	WATES	Do you do anything to make the water safer to drink?	☐ Boil ☐ Add Bleach/Chlorine/ Water Guard ☐ Strain through a cloth ☐ Use water filter (ceramic/sand/etc) ☐ Solar disinfection ☐ Let it stand and settle ☐ Other (specify on Form Z) ☐ No, Nothing ☐ Don't know
E27	WC	What is the principle type of toilet facility used by household members?	☐ Own (exclusive) flush ☐ Shared flush toilet ☐ Ventilated (VIP) latrine ☐ Pit latrine with slab ☐ Pit laterine without slab/open pit ☐ Bush or field ☐ Other ☐ Dont know
E28	WCNO	How many households, including yours, use this toilet facility?	☐☐ 99 = Don't Know

E29	PID	Patient ID	☐☐☐☐ - ☐ A/B/C
E30	PIN	Patient Initials	☐☐

E31	FLOR	What is the principle type of flooring in your dwelling?	☐ Tiles/cement/carpet/vinyl (1) ☐ Wood or planks or brocken bricks (2) ☐ Dirt, sand or dung (3) ☐ Other (9) (specify on Z) ☐ Dont know (99)
E32	ROOF	What is the principle type of roofing in your dwelling?	☐ Cement (1) ☐ Wood planks or Cardboard (2) ☐ Iron and tiles (3) ☐ Iron sheets (4) ☐ Natural materials (palm/thatched) (5) ☐ Other (9) ☐ No roof (6) ☐ Dont know (99)
E33	BANK	Do any members of the household have a bank account?	☐ Yes ☐ No ☐ Don't Know
E34	SRAY	In the past 12 months, has anyone come into your house to spray the inside walls against mosquitos?	☐ Yes ☐ No ☐ Don't Know
E35	NETS	How many mosquito nets that can be used while sleeping does your house have?	☐☐ 00 = NONE 99 = Don't know

E36 PID Patient ID ☐☐☐☐ - ☐ A/B/C

E37 PIN Patient Initials ☐☐

E38 SMOK Does anyone ever smoke tobacco in the home?

- ☐ Daily (1)
- ☐ Weekly (2)
- ☐ Monthly (3)
- ☐ Less than once a month (4)
- ☐ Never (5) *If never, skip to Question E40*

E39 SMOKW If yes, who? (tick all that apply)

- ☐ Mother(1)
- ☐ Father(2)
- ☐ Grandparent (3)
- ☐ Sibling (4)
- ☐ Child (study) (5)
- ☐ Other (6)

E40 MSMOK Did the mother smoke during preganacy? ☐ Yes ☐ No ☐ Dont know

E41 COOK What type of fuel does your household MAINLY use for cooking?

- ☐ Electricity (1)
- ☐ LPG/Natural Gas (2)
- ☐ Biogas (3)
- ☐ Kerosene (4)
- ☐ Coal, Lignite (5)
- ☐ Charcoal (6)
- ☐ Wood (7)
- ☐ Staw/Shrubs/Grass (8)
- ☐ Animal Dung (9)
- ☐ Other (10) (Specify on Z)

E42 STOV What type of stove/fire is used for cooking?

- ☐ Open fire (1)
- ☐ Stove without chimney/flute (2)
- ☐ Stove with chimney/flute (3)

E43 COOKIN Where is the cooking usually done?

- ☐ In the house (1)
- ☐ In a separate building(2)
- ☐ Outside (3)

Form K. Family Health History. ChroSAM study.

K1	PID	Participant ID	☐☐☐☐ - ☐ A/B/C
K2	PIN	Participant Initials	☐☐
K3	INTDT	Interview Date	☐☐ / ☐☐☐ / 2 0 ☐☐ (D D / M O N / Y Y Y Y)
K4	OPIN	Operator Initials	☐☐

Participant ID = HMIS number followed by
A –moyo child
B –si**B**ling control
C –**C**ommunity conrol

Mother HTC

K5 MHIVt — Tested for HIV?

- ☑ Never Tested (2)
- ☐ Tested (1)
- ☐ Don't Know (99)
- ☐ Prefer not to say (88)

K6 MHIV — If test – HIV status?

- ☐ R (positive) (1)
- ☐ NR (Negative) (2)
- ☐ Dont know (99)
- ☐ Prefer not to say (88)

If NR, skip to K8

If not tested, note why on Master form Z

K7 MARV — Is mother taking ARVs?

- ☐ No (2)
- ☐ Yes (1)
- ☐ On waiting list (3)
- ☐ Not eligible (4)
- ☐ Started but now stopped (5)
- ☐ Don't know (99)

K8 MHLT — Has mother been very sick for atleast 3 months in past year? ie. too sick to work or do normal activities?

☐ Yes ☐ No ☐ Dont know

Specify Any other health problems on Master form z

K10 Participant ID ☐☐☐☐ - ☐

K11 Participant Initials ☐☐

Father HTC

K12 FHIVt Tested for HIV?

- ☐ Never Tested (2)
- ☐ Tested (1)
- ☐ Don't Know (99)
- ☐ Prefer not to say (88)

K13 FHIV If test, HIV status

- ☐ R (positive) (1)
- ☐ NR (negative) (2)
- ☐ Dont know (99)
- ☐ Prefer not to say (88)

If NR, skip to K15

If not tested, note why on Master form Z

K14 FARV Is Father taking ARVs?

- ☐ No (2)
- ☐ Yes (1)
- ☐ On waiting list (3)
- ☐ Not eligible (4)
- ☐ Started but now stopped (5)
- ☐ Don't know (99)

K15 FHLT Has Father been very sick for atleast 3 months in past year? ie. too sick to work or do normal activities?

☐ Yes ☐ No ☐ Dont know

Specify any other health problems on Master form Z

K16	PID	Participant ID ☐☐☐☐ - ☐
K17	PIN	Participant Initials ☐☐

First Born –Health
(unless Moyo or control child)

Is this the Moyo or control child? ☐ Yes ☐ No

If yes, skip to next child

K18 SAM1 Ever had Kwash or Marasmus?

- ☐ Never (2)
- ☐ NRU admission (1)
- ☐ CTC (3)
- ☐ Given multivit/ drugs only (4)
- ☐ Advice only (5)
- ☐ Nil Rx (6)
- ☐ SFP (7)

K19 HOS1 Number of hospital admissions in last year ☐☐

Note any other health problems or hospital admissions details on Master Form Z

2nd Born –Health
(unless Moyo or control child)

Is this the Moyo or control child? ☐ Yes ☐ No

If yes, skip to next child

K20 SAM2 Ever had Kwash or Marasmus?

- ☐ Never (2)
- ☐ NRU admission (1)
- ☐ CTC (3)
- ☐ Given multivit/ drugs only (4)
- ☐ Advice only (5)
- ☐ Nil Rx (6)
- ☐ SFP (7)

K21 HOS2 Number of hospital admissions in last year ☐☐

Note any other health problems or hospital admissions details on Master Form Z

K22 PID Participant ID ☐☐☐☐ - ☐

K23 PIN Participant Initials ☐☐

3rd Born –Health
(unless Moyo or control child)

Is this the Moyo or control child? ☐ Yes ☐ No

If yes, skip to next child

K24 SAM3 Ever had Kwash or Marasmus?

- ☐ Never (2)
- ☐ NRU admission (1)
- ☐ CTC (3)
- ☐ Given multivit/ drugs only (4)
- ☐ Advice only (5)
- ☐ Nil Rx (6)
- ☐ SFP (7)

K25 HOS3 Number of hospital admissions in last year ☐☐

Note any other health problems or hospital admissions details on Master Form Z

4th Born –Health
(unless Moyo or control child)

Is this the Moyo or control child? ☐ Yes ☐ No

If yes, skip to next child

K26 SAM4 Ever had Kwash or Marasmus?

- ☐ Never
- ☐ NRU admission
- ☐ CTC
- ☐ Given multivit/ drugs only
- ☐ Advice only
- ☐ Nil Rx
- ☐ SFP

K27 HOS4 Number of hospital admissions in last year ☐☐

Note any other health problems or hospital admissions details on Master Form Z

K28 PID Participant ID ☐☐☐☐ - ☐

K29 PIN Participant Initials ☐☐

5th Born –Health
(unless Moyo or control child)

Is this the Moyo or control child? ☐ Yes ☐ No

If yes, skip to next child

K30 SAM5 Ever had Kwash or Marasmus?

- ☐ Never (2)
- ☐ NRU admission (1)
- ☐ CTC (3)
- ☐ Given multivit/ drugs only (4)
- ☐ Advice only (5)
- ☐ Nil Rx (6)
- ☐ SFP (7)

K31 HOS5 Number of hospital admissions in last year ☐☐

Note any other health problems or hospital admissions details on Master Form Z

6th Born –Health
(unless Moyo or control child)

Is this the Moyo or control child? ☐ Yes ☐ No

If yes, skip to next child

K32 SAM6 Ever had Kwash or Marasmus?

- ☐ Never
- ☐ NRU admission
- ☐ CTC
- ☐ Given multivit/ drugs only
- ☐ Advice only
- ☐ Nil Rx
- ☐ SFP

K33 HOS6 Number of hospital admissions in last year ☐☐

Note any other health problems or hospital admissions details on Master Form Z

K34 PID Participant ID ☐☐☐☐ - ☐

K35 PIN Participant Initials ☐☐

7th Born –Health
(unless Moyo or control child)

Is this the Moyo or control child? ☐ Yes ☐ No

If yes, skip to next child

K36 SAM7 Ever had Kwash or Marasmus?

- ☐ Never (2)
- ☐ NRU admission (1)
- ☐ CTC (3)
- ☐ Given multivit/ drugs only (4)
- ☐ Advice only (5)
- ☐ Nil Rx (6)
- ☐ SFP (7)

K37 HOS7 Number of hospital admissions in last year ☐☐

Note any other health problems or hospital admissions details on Master Form Z

8th Born –Health
(unless Moyo or control child)

Is this the Moyo or control child? ☐ Yes ☐ No

If yes, skip to next child

K38 SAM8 Ever had Kwash or Marasmus?

- ☐ Never (2)
- ☐ NRU admission (1)
- ☐ CTC (3)
- ☐ Given multivit/ drugs only (4)
- ☐ Advice only (5)
- ☐ Nil Rx (6)
- ☐ SFP (7)

K39 HOS8 Number of hospital admissions in last year ☐☐

Note any other health problems or hospital admissions details on Master Form Z

Appendix 8: Maternal mental health SRQ

ChroSAM FORM F: SELF REPORTING QUESTIONNAIRE

Participant ID: …… Participant Initials (child)……

Tsopano ndikufunsani mafunso okhudzana ndi momwe mumamvera mumtima ndi maganizo omwe mwakhala nawo m'sabata zinayi zomwe zapitazi. Muyankhe "eya" kapena "ayi" ku funso lirilonse. Ngati mukukaikira, yankhani mofanizira ndi momwe mwakhala mukumvera. Ngati simukumvetsa funso, chonde funsani ndipo ndikupatsani chitsanzo chotanthauzira funsolo

1	M'masabata anayi apitawa, kodi mumamva kupweteka mutu pafupipafupi?	❒ [1] Eya ❒ [0] Ayi
2	M'masabata anayi apitawa, kodi simumakhala ndi chilakolako cha chakudya?	❒ [1] Eya ❒ [0] Ayi
3	M'masabata anayi apitawa, kodi mumavutika kugona usiku?	❒ [1] Eya ❒ [0] Ayi
4	M'masabata anayi apitawa, kodi manja anu amanjenjemera?	❒ [1] Eya ❒ [0] Ayi
5	M'masabata anayi apitawa, kodi mumakhala ndi nkhawa, mantha kapena madandaulo?	❒ [1] Eya ❒ [0] Ayi
6	M'masabata anayi apitawa, kodi simumachedwa kututumutsidwa?	❒ [1] Eya ❒ [0] Ayi
7	M'masabata anayi apitawa, kodi mumadzimbidwadzimbidwa?	❒ [1] Eya ❒ [0] Ayi
8	M'masabata anayi apitawa, kodi mumakhala ndi vuto kuganiza bwinobwino?	❒ [1] Eya ❒ [0] Ayi
9	M'masabata anayi apitawa, kodi mumakhala osasangalala kapena osakondwa?	❒ [1] Eya ❒ [0] Ayi
10	M'masabata anayi apitawa, kodi mumaliralira pafupipafupi ndipo koposera muyeso?	❒ [1] Eya ❒ [0] Ayi
11	M'masabata anayi apitawa, kodi mumaona ngati ndi chinthu chokuvutani kusangalatsidwa ndi zinthu zimene mumapanga tsiku ndi tsiku?	❒ [1] Eya ❒ [0] Ayi
12	M'masabata anayi apitawa, kodi mumakhala ndi vuto kupanga maganizo kapena kumanga mfundo?	❒ [1] Eya ❒ [0] Ayi
13	M'masabata anayi apitawa, kodi ntchito zanu za tsiku ndi tsiku sizimayenda bwino?	❒ [1] Eya ❒ [0] Ayi
14	M'masabata anayi apitawa, kodi mumalephera kupanga zinthu za phindu kapena zofunikira m'moyo wanu?	❒ [1] Eya ❒ [0] Ayi
15	M'masabata anayi apitawa, kodi munasiya kukhala ndi chidwi mu zinthu zosiyanasiyana?	❒ [1] Eya ❒ [0] Ayi
16	M'masabata anayi apitawa, kodi mumaziona ngati ndinu munthu wopanda ntchito kapena wosafunikira?	❒ [1] Eya ❒ [0] Ayi
17	M'masabata anayi apitawa, kodi maganizo odzipha anayamba akubwereranipo? *	❒ [1] Eya ❒ [0] Ayi
18	M'masabata anayi apitawa, kodi mumamva kapena kukhala otopatopa nthawi zonse?	❒ [1] Eya ❒ [0] Ayi
19	M'masabata anayi apitawa, kodi mumakhala ndi vuto losamva bwino m'mimba?	❒ [1] Eya ❒ [0] Ayi
20	M'masabata anayi apitawa, kodi simumachedwa kutopa?	❒ [1] Eya ❒ [0] Ayi

** IMPORTANT- If the mother answers yes, at the end of the interview, ask her more about these thoughts and refer to counseling services. Does she have them all the time? Has she thought of a method of hurting herself? Has she actually tried to hurt or even kill herself?* **(If YES, call Dr Ahrens or Dr Bosnak 0996604636)**

Maternal Mental Health: SELF REPORTING QUESTIONNAIRE - ENGLISH

Now I am going to ask you some questions about the thoughts and feelings that you have experienced over the last 4 weeks. You should answer yes or no to each question. If you are not sure, give the answer that is closest to how you have been feeling. If you do not understand a question please ask and I can give you an example of what the question means.

1	Do you often have headaches?	Yes	No
2	Is your appetite poor?	Yes	No
3	Do you sleep badly?	Yes	No
4	Do your hands shake?	Yes	No
5	Do you feel nervous tense or worried?	Yes	No
6	Are you easily frightened?	Yes	No
7	Is your digestion poor?	Yes	No
8	Do you have trouble thinking clearly?	Yes	No
9	Do you feel unhappy?	Yes	No
10	Do you cry more than usual?	Yes	No
11	Do you find it difficult to enjoy your daily activities?	Yes	No
12	Do you find it difficult to make decisions?	Yes	No
13	Is your daily work suffering?	Yes	No
14	Are you unable to play a useful part in life?	Yes	No
15	Have you lost interest in things?	Yes	No
16	Do you feel that you are a worthless person?	Yes	No
17	Has the thought of ending your life been on your mind?	Yes	No
18	Do you feel tired all the time?	Yes	No
19	Do you have uncomfortable feelings in your stomach?	Yes	No
20	Are you easily tired?	Yes	No

Appendix 9: Disability screening questionnaire

B. Unicef/Washington Group Questions (to be reported by parents)

Is the child 2 years or above? Yes O No O

If YES, Proceed below, if NO, SKIP to Section D (Rehab)

Children aged 2-17 years

1a) Does [name] wear glasses or contact lenses Yes O No O

	No difficulty	Some difficulty	A lot of difficulty	Cannot do at all	Dont Know	Refused
[if child wears glasses] 1b) Does [name] have difficulty seeing, when wearing his/her glasse	1	2	3	4	5	6
[if child does NOT wear glasses] 1c) Does [name] have difficulty seeing?	1	2	3	4	5	6

Children aged 2-17 years

2a) Does [name] use a hearing aid? Yes O No O

If YES go to question 2b

If NO go to question 2c

	No difficulty	Some difficulty	A lot of difficulty	Cannot do at all	Dont Know	Refused
[if child uses a hearing aid] 2b) Does [name] have difficulty hearing, when using his/her hearing a	1	2	3	4	5	6
[if child does NOT use a hearing aid] 2c) Does [name] have difficulty hearing?	1	2	3	4	5	6

	No difficulty	Some difficulty	A lot of difficulty	Cannot do at all	Dont Know	Refused
Children aged 2-17 years						
3) Compared with children of the same age, does [name] have difficulty walking?	1	2	3	4	5	6
Children aged 5- 17 years						
4) Compared with children of the same age, does [name] have difficulty with self-care such as feeding or dressing him/herself?	1	2	3	4	5	6
Children aged 2 -4 years						
5a) Does [name] have difficulty understanding you?	1	2	3	4	5	6
6a) Do you have difficulty understanding what your child wants?	1	2	3	4	5	6
Children aged 5-17 years						
5b) Compared with children of the same age and using [his/her] usual language, does [name] have difficulty understanding other people?	1	2	3	4	5	6
6b)Compared with children of the same age and using [his/her] usual language, does [name] have difficulty being understood by other people?	1	2	3	4	5	6
Children aged 2-3 years						
7a) Compared with children of the same age, does [name] have difficulty learning the names of common objects?	1	2	3	4	5	6
Children aged 3-17 years						
7b) Compared with children of the same age, does [name] have difficulty learning to do new things?	1	2	3	4	5	6
Children aged 5-17 years						
8) Compared with children of the same age, does [name] have difficulty remembering things that they have learned?	1	2	3	4	5	6

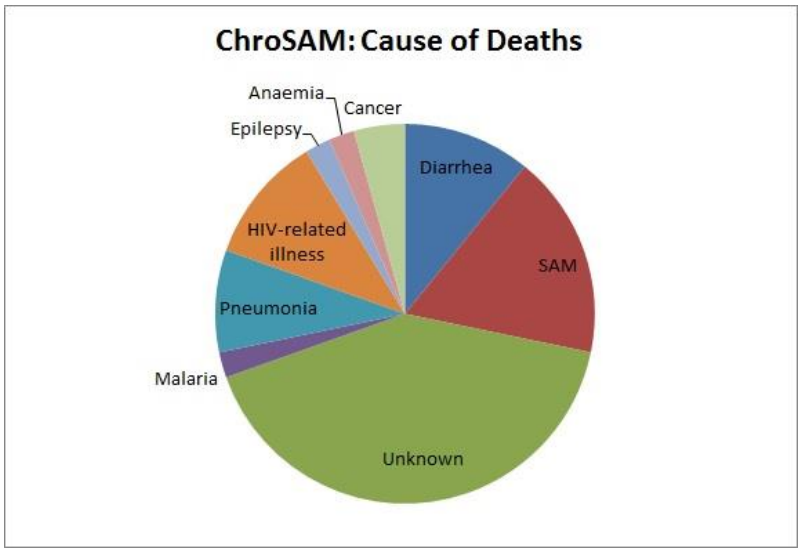
	Less	The same	More	A lot more	Dont Know	Refused
Children aged 5-17 years						
9) Compared with children of the same age, how much does [he /she] worry or feel sad?	1	2	3	4	5	6
Children aged 2-4 years						
10a) Compared with children of the same age, how much does [name] kick, bite or hit other children or adults? (Either provoked or unprovoked)	1	2	3	4	5	6

	No difficulty	Some difficulty	A lot of difficulty	Cannot do at all	Dont Know	Refused
Children aged 5-17 years						
10b) Compared with children of the same age, how much difficulty does [name] have controlling [his/her] behaviour?	1	2	3	4	5	6
11) Compared with children of the same age, does [name] have difficulty completing a task?	1	2	3	4	5	6
12) Compared with children of the same age, does [name] have difficulty accepting change to plans or routine?	1	2	3	4	5	6
13) Does [name] have difficulty getting along with children of [his/her] age?	1	2	3	4	5	6
Children aged 2-5 years						
14 a1) Compared with children of the same age, does [name] have difficulty playing with toys or household objects?	1	2	3	4	5	6
Children aged 2-12 years						
14a2) Compared with children of the same age, does [name] have difficulty playing with other children?	1	2	3	4	5	6
Children aged 13-17 years						
14b) Compared with children of the same age, does [name] have difficulty doing things with other children? (Include things that children usually do together.)	1	2	3	4	5	6

Appendix 10: Validating wealth quintiles; graph of mean HAZ for each quintile

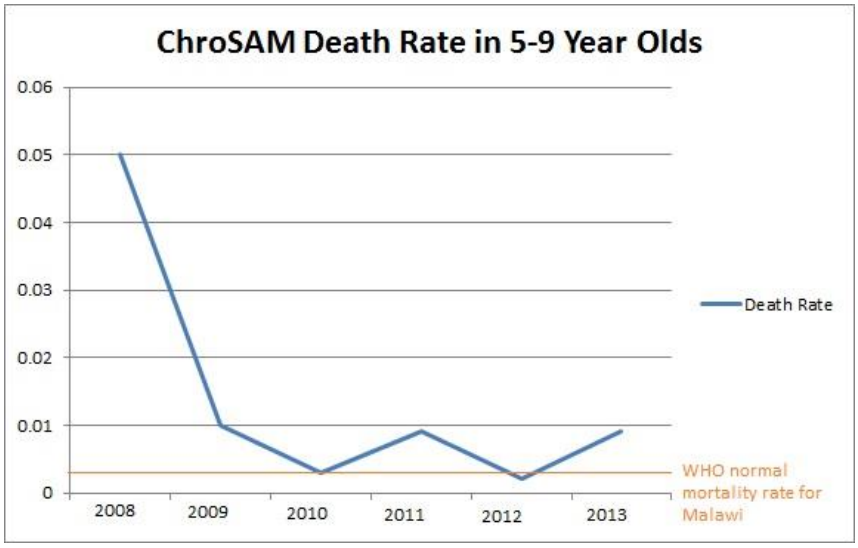
Appendix 10: graph showing mean height-for-age z score (HAZ) for children in each of the 5 wealth quintiles. Wealth quintiles were devised from asset data using Principle component analysis (PCA); quintile 1 in lowest socioeconomic status and quintile 5 is the highest

Appendix 11: Pie chart showing cause of death for 46 ChroSAM deaths



Appendix 11: pie chart showing cause of death for 46 "ChroSAM" deaths

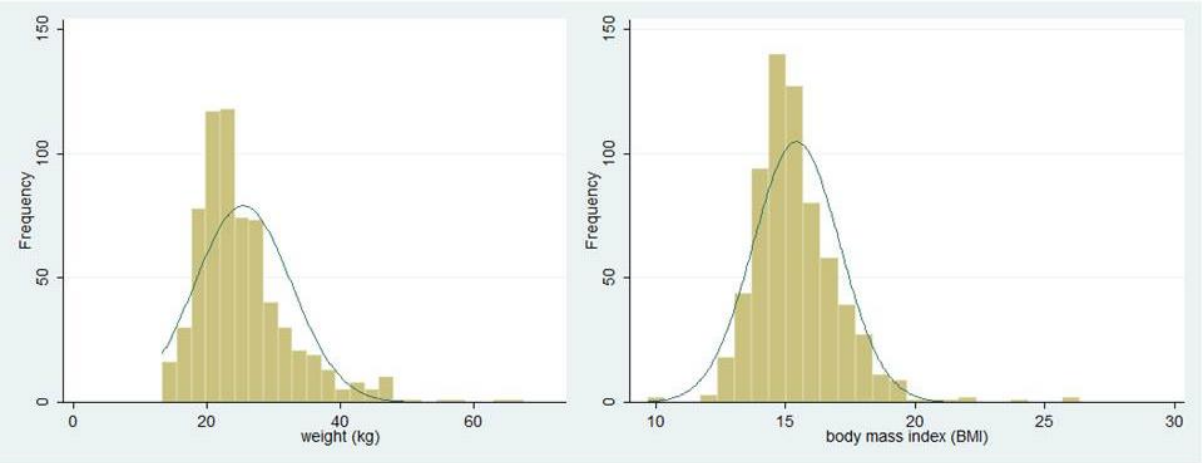
Appendix 12: Line graph showing mortality rate across ChroSAM follow up period



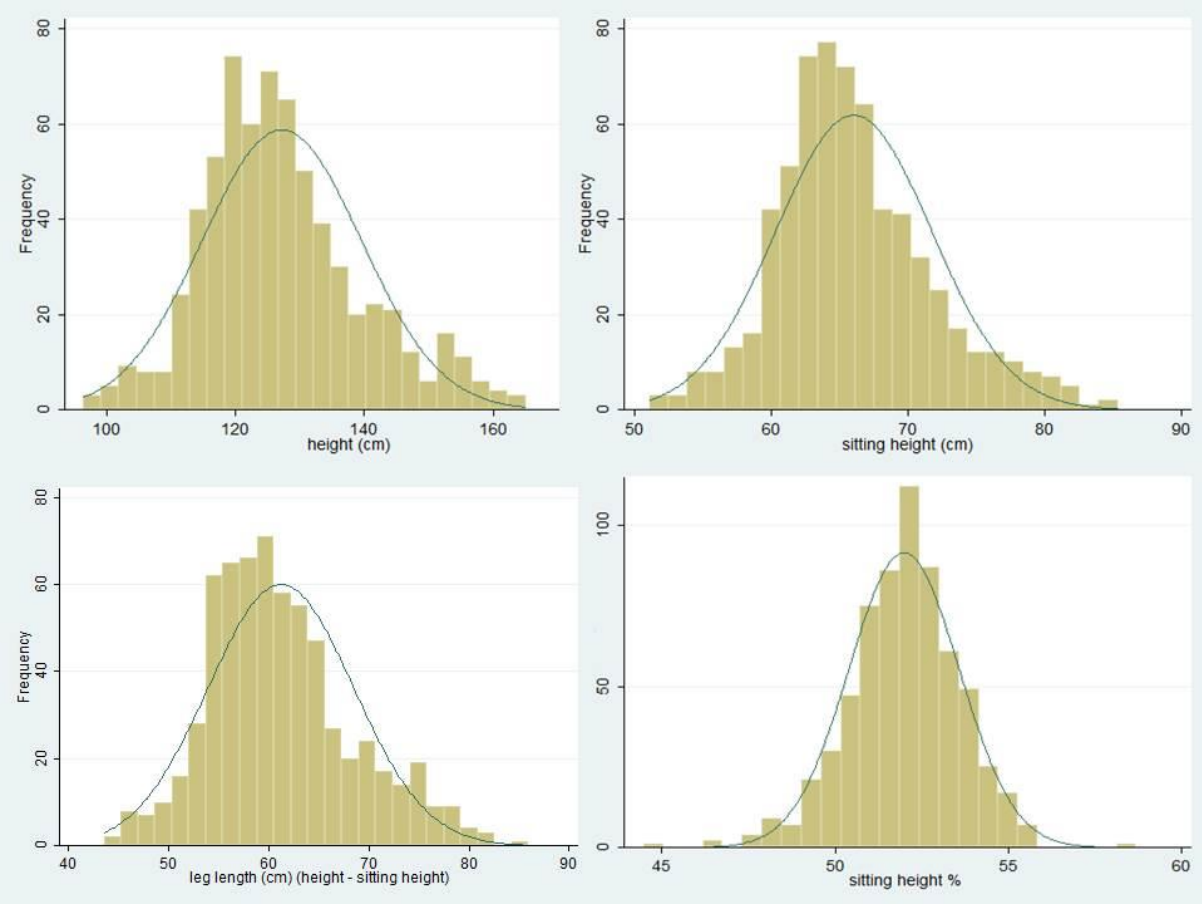
Appendix 12: death rate across ChroSAM follow-up period, with line in orange showing WHO life tables normal rate for this age group (5-9 years) in Malawi

Appendix 13: Histograms of growth outcomes

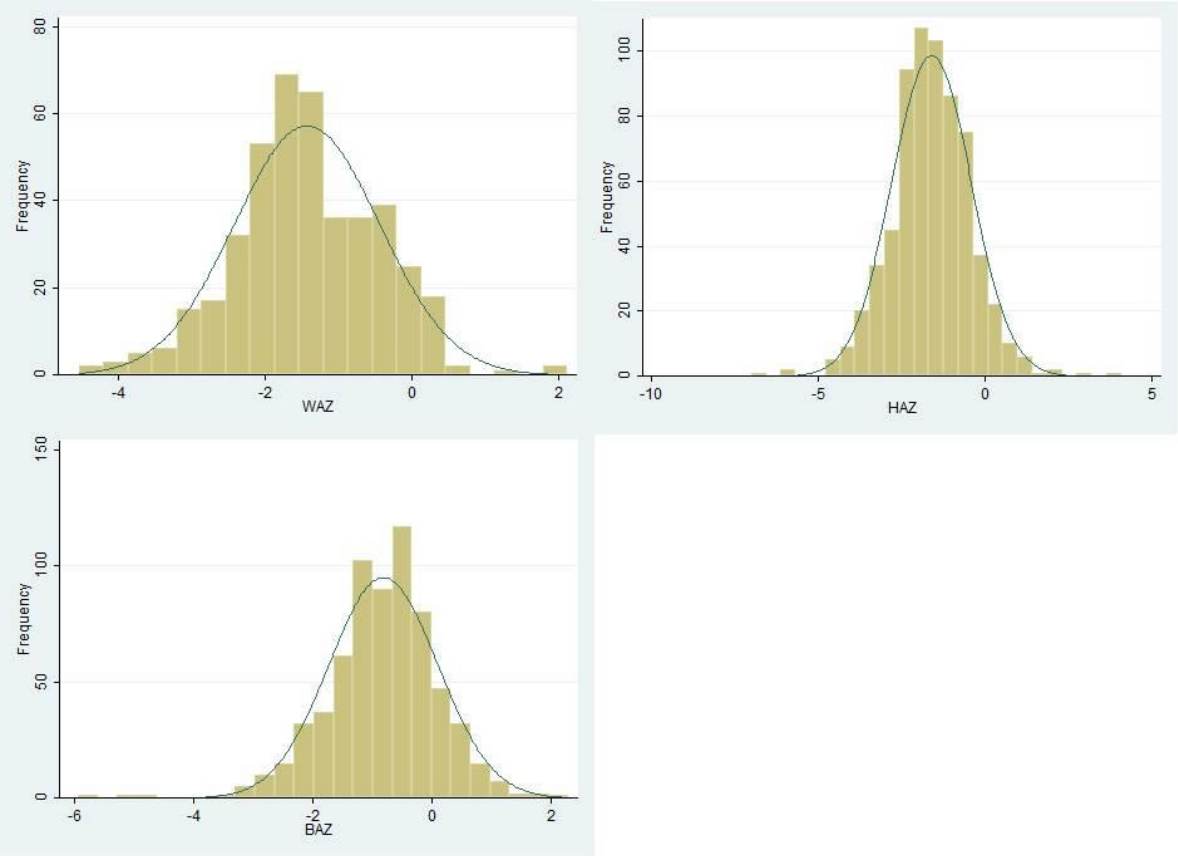
Weight



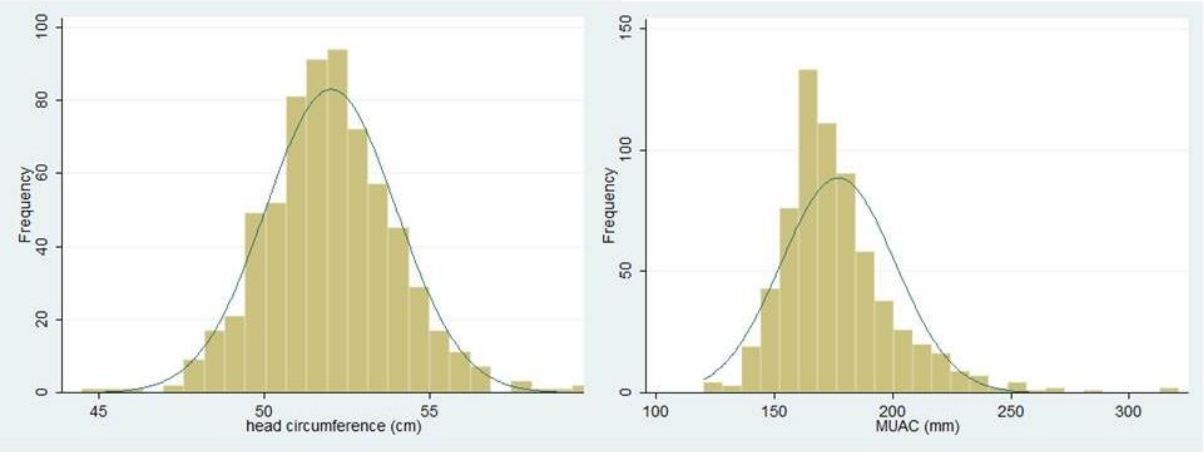
Height



Growth z scores



Body Circumferences

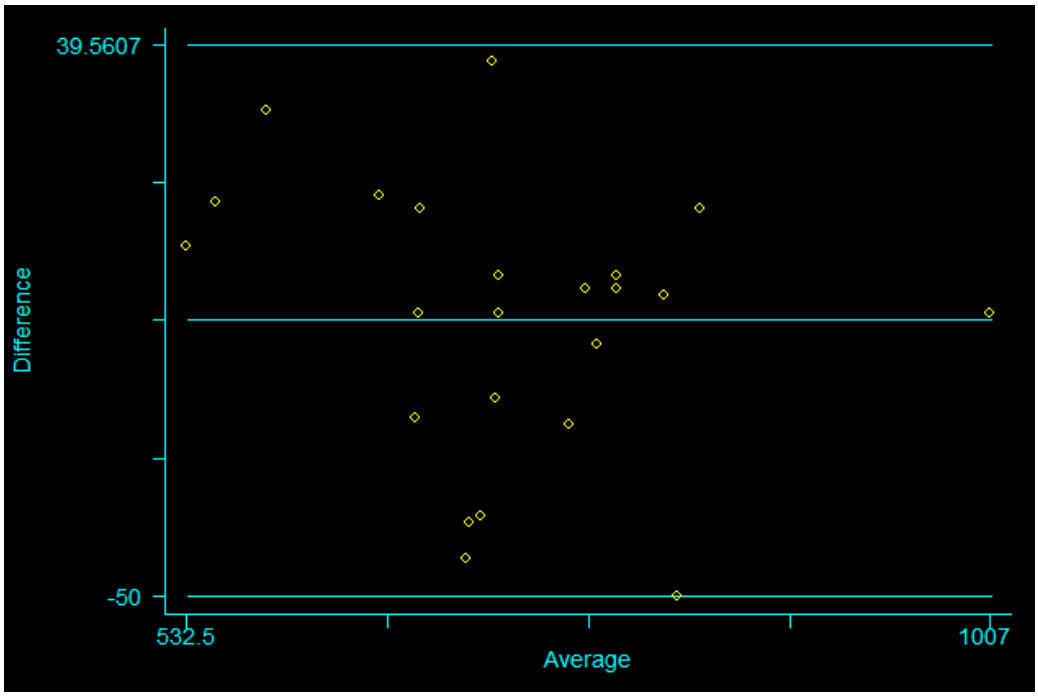


Appendix 14: Table of results comparing growth outcomes for HIV negative children only

Anthropometry	HIV- Cases	HIV- Sibling			HIV- Community		
	Mean (SD)	Mean (SD)	Unadjusted difference (95% CI)	Adjusted difference (95% CI)	Mean (SD)	Unadjusted difference (95% CI)	Adjusted difference (95% CI)
Weight							
Weight (kg)	24.0 (5.0)	28.0 (9.7)	4.0 * (2.5, 5.4)	2.0 * (1.1, 3.0)	25.4 (6.6)	1.4 (-0.1, 2.9)	1.5 * (0.6, 2.5)
BMI	15.2 (1.5)	15.8 (1.9)	0.6 * (0.3, 0.9)	0.3 * (0.1, 0.6)	15.4 (1.7)	0.2 (-0.2, 0.5)	0.2 (-0.1, 0.5)
Height							
Standing (cm)	125 (8.7)	130.7 (16.8)	5.5 * (3.0, 7.9)	2.1 * (0.7, 3.5)	127.4 (10.1)	2.2 (-0.4, 4.7)	2.5 * (1.0, 4.0)
Sitting (cm)	65.3 (4.3)	67.6 (7.6)	2.3 * (1.1, 3.4)	0.8 * (0.0, 1.5)	66.0 (4.6)	0.7 (-0.5,1.9)	0.8 * (0.0, 1.6)
Sitting Height %	52.2 (1.5)	51.8 (1.7)	-0.4 * (-0.7, -0.1)	-0.2 (-0.5, 0.0)	51.8 (1.4)	-0.4 * (-0.7,-0.1)	-0.5 * (-0.8,-0.2)
Leg length (cm)	60 (5.4)	63.2 (9.7)	3.2 * (1.7, 4.6)	1.4 * (0.4, 2.3)	61.5 (6.0)	1.5 (-0.1, 3.0)	1.7 * (0.7, 2.7)
Relative leg length	40.5 (17)	50.5 (32.9)	10.0 * (5.2, 14.8)	3.5 * (0.6, 6.5)	44.1 (19.4)	3.6 (-1.5, 8.8)	4.1 * (1.0,7.2)
Lower limb length ratio	31.0 (1.7)	31.7 (1.7)	0.6 * (0.3, 1.0)	0.5 * (0.2, 0.8)	31.4 (1.5)	0.4 * (0.0, 0.7)	0.4 * (0.1, 0.7)
Growth Z scores							
WAZ	-1.5 (0.9)	-1.4 (1.0)	0.1 (-0.1, 0.4)	-0.2 (-0.5, 0.1)	-1.2 (1.0)	0.3 * (0.1, 0.5)	0.2 * (0.0, 0.5)
HAZ	-1.7 (1.2)	-1.5 (1.2)	0.2 (-0.0, 0.4)	0.2 * (0.0, 0.5)	-1.3 (1.1)	0.4 * (0.2, 0.7)	0.4 * (0.1, 0.6)
BAZ	-0.8 (1,0)	-0.8 (0.9)	0.03 (-0.2, 0.2)	0.08 (-0.1, 0.2)	-0.7 (0.9)	0.14 (-0.1, 0.3)	0.11 (-0.1, 0.3)
Body circumferences							
Head (cm)	51.8 (1.9)	52.2 (2.2)	0.4 * (0.0, 0.8)	0.2 (-0.1, 0.6)	52.1 (1.9)	0.3 (-0.1, 0.7)	0.4 * (0.0, 0.7)
MUAC (mm)	172 (19)	184 (30)	12.3 * (7.6, 17.0)	7.0 * (3.5, 10.5)	178 (22)	6.5 * (1.5, 11.5)	6.6 * (2.9, 10.3)

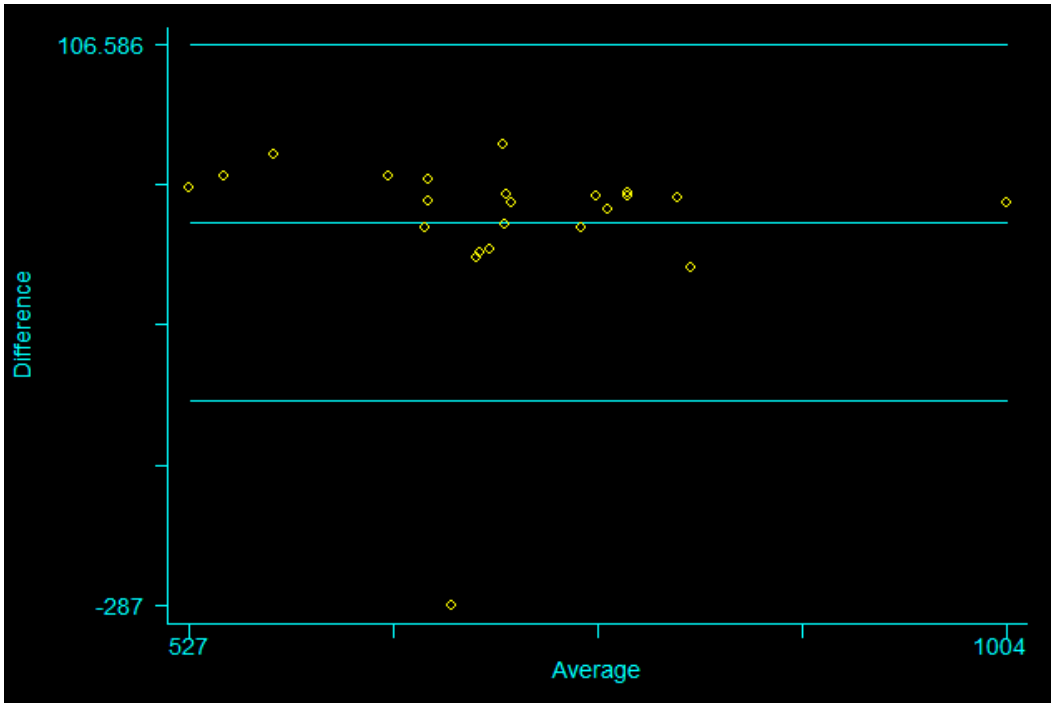
Appendix 15: Bland-Altman plots of "agreement" for repeat BIA readings

Impedance:



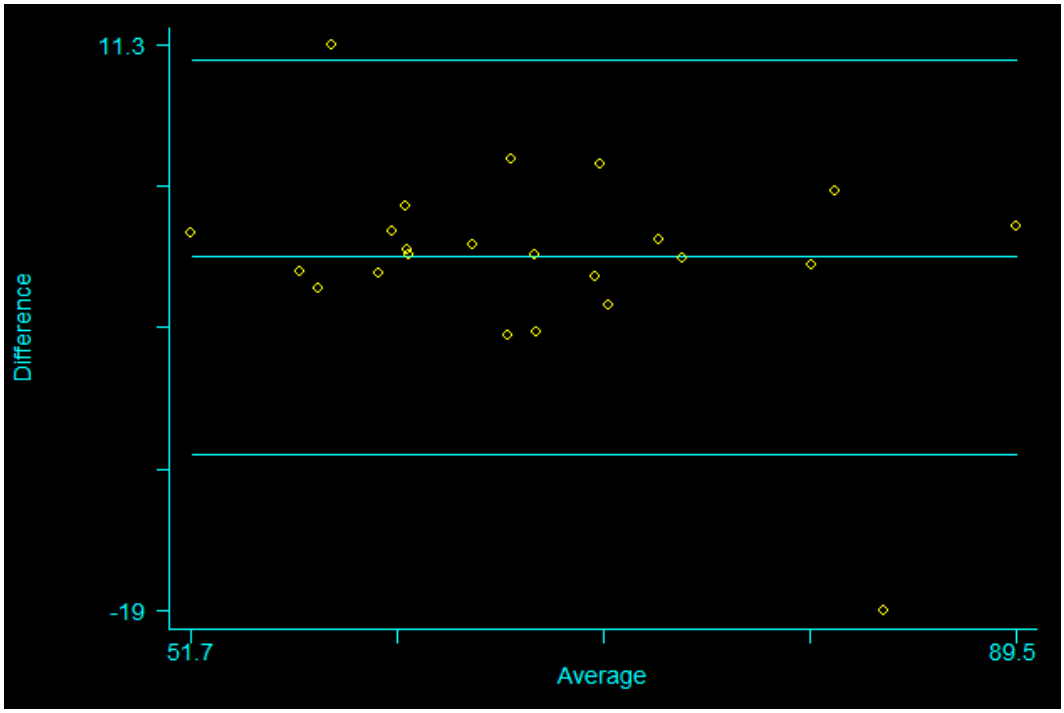
Bland-Altman plot for Impedance: Mean difference is 5.2 (middle line); limits of agreement (±2 SD from mean difference) is -50.0 to 39.6 (top and bottom line). 95% of data plots should lie within limits of agreement.

Resistance:



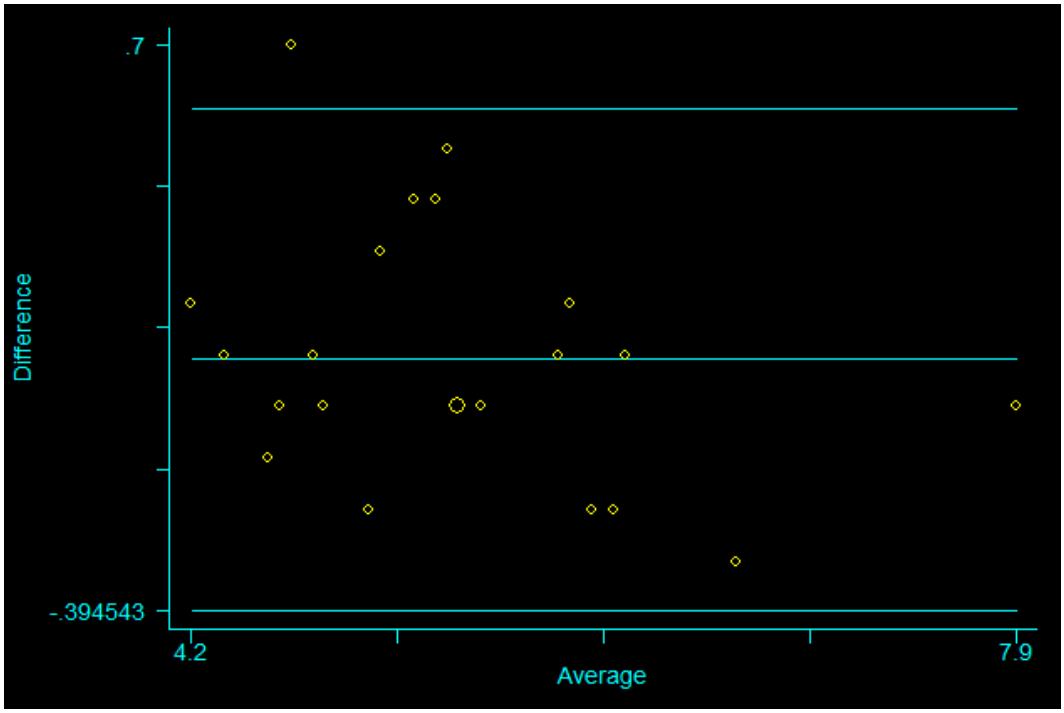
Bland-Altman plot for Resistance: Mean difference is 18.4 (middle line); limits of agreement (±2 SD from mean difference) is -143.0 to 106.6 (top and bottom line). 95% of data plots should lie within limits of agreement.

Reactance:



Bland-Altman plot for Reactance: Mean difference is 0.06 (middle line); limits of agreement (±2 SD from mean difference) is -10.6 to 10.5 (top and bottom line). 95% of data plots should lie within limits of agreement.

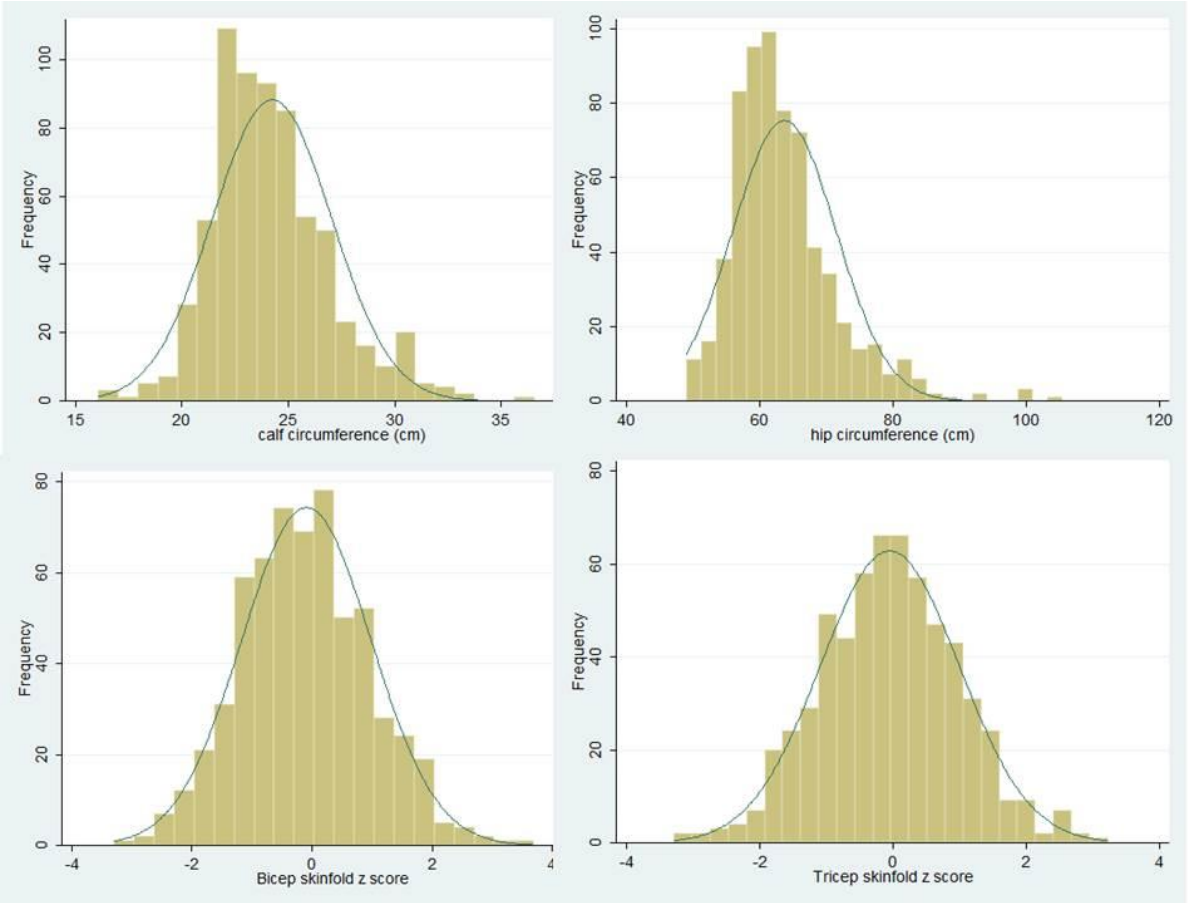
Phase Angle:



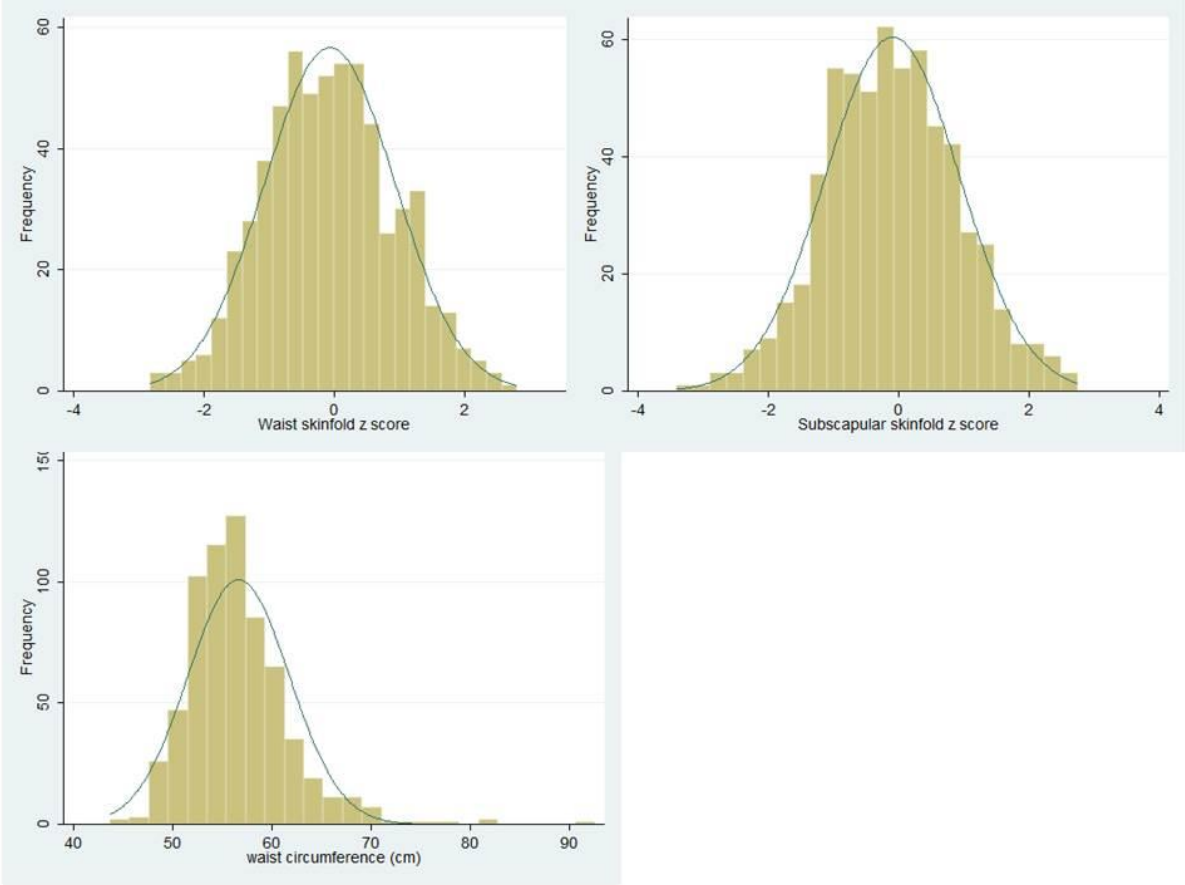
Bland-Altman plot for Phase Angle: Mean difference is -0.09 (middle line); limits of agreement (±2 SD from mean difference) is -0.4 to 0.6 (top and bottom line). 95% of data plots should lie within limits of agreement.

Appendix 16: Histograms of Body composition outcomes

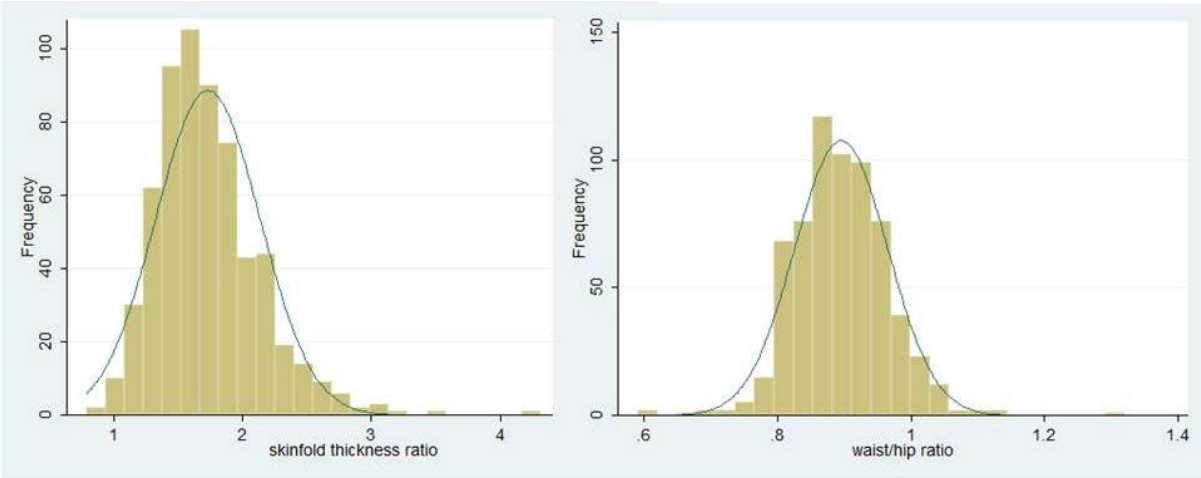
Peripheral fat



Core Fat



Ratios of Core to Peripheral Fat



Appendix 17: Table of results comparing body composition outcomes for HIV negative children only

Anthropometry	Cases	Sibling			Community		
	Mean (SD)	Mean (SD)	Unadjusted difference (95% CI)	Adjusted difference (95% CI)	Mean (SD)	Unadjusted difference (95% CI)	Adjusted difference (95% CI)
Peripheral fat							
Calf circumference (cm)	23.7 (2.3)	25.1 (3.5)	1.44 * (0.9, 2.0)	0.80 * (0.4, 1.2)	24.3 (2.4)	0.62 * (0.0, 1.2)	0.66 * (0.2, 1.1)
Hip circumference (cm)	62.4 (5.8)	65.9 (10.0)	3.49 * (2.0, 5.0)	1.57 * (0.5, 2.6)	63.8 (7.0)	1.32 (-0.3, 3.0)	1.28 * (0.2, 2.4)
Relative hip circumference	0.50 (0.0)	0.50 (0.0)	0.01 (-0.0, 0.0)	0.0 (-0.0, 0.01)	0.50 (0.0)	0.00 (-0.0, 0.0)	0.00 (-0.0,0.0)
Tricep skinfold z score	-0.07 (1.1)	0.10 (0.9)	0.17 (-0.0, 0.4)	0.15 (-0.1, 0.3)	-0.12 (1.1)	-0.04 (-0.3,0.2)	-0.04 (-0.3, 0.2)
Bicep skinfold z score	-0.23 (1.1)	0.05 (1.0)	0.29 * (0.1,0.5)	0.25 * (0.0, 0.5)	-0.05 (1.0)	0.18 (-0.0,0.4)	0.16 (-0.1, 0.4)
Core fat							
Waist circumference (cm)	56.2 (4.2)	57.8 (6.5)	1.65 * (0.6, 2.7)	0.64 (-0.2, 1.4)	56.0 (4.6)	-0.13 (-1.2, 1.0)	0.08 (-0.8, 0.9)
Relative waist circumference	0.45 (0.0)	0.44 (0.0)	-0.00 (-0.01, 0.0)	-0.00 (-0.0, 0.0)	0.44 (0.0)	-0.01 * (-0.01, -0.0)	-0.01 * (-0.1,-0.0)
Waist skinfold z score	-0.17 (1.0)	0.16 (1.0)	0.33 * (0.1,0.5)	0.31 * (0.1, 0.5)	-0.17 (1.0)	0.01 (-0.2, 0.2)	-0.02 (-0.2, 0.2)
Subscapular skinfold z score	-0.23 (1.1)	0.12 (1.0)	0.35 * (0.1, 0.6)	0.32 * (0.1, 0.5)	-0.16 (1.0)	0.08 (-0.1, 0.3)	0.06 (-0.2, 0.3)
Ratio of core to peripheral fat							
Skinfold thickness ratio	1.7 (0.4)	1.8 (0.4)	0.07 (-0.0, 0.1)	0.04 (-0.0, 0.1)	1.7 (0.4)	0.03 (-0.1, 0.1)	0.03 (-0.1, 0.1)
Waist/hip ratio	0.90 (0.1)	0.88 (0.1)	-0.02 * (-0.03, -0.0)	-0.01 (-0.02, 0.0)	0.89 (0.1)	-0.02 * (-0.03, -0.0)	-0.02 * (-0.0,-0.0)
Lean Mass Index							
1/z (lean mass)	9.07 (1.1)	9.22 (1.0)	0.15 (-0.1,0.3)	0.22 * (0.02, 0.4)	9.13 (1.0)	0.06 (-0.2, 0.3)	0.07 (-0.1,0.3)
BMI residual from 1/Z (fat mass)	-0.20 (1.2)	0.98 (1.8)	0.49 * (0.2, 0.8)	0.18 (-0.1, 0.4)	-0.09 (1.6)	0.12 (-0.2, 0.4)	0.12 (-0.2, 0.4)
BIVA							
r/h	599 (100)	563 (128)	-36.3 * (-58, -15)	-16.7 (-34, 0.3)	577 (91.8)	-21.9 (-45, 1.1)	-24.5 * (-43,-6.5)
xc/h	51.3 (7.8)	49.3 (9.1)	-2.1 * (-3.7,-0.4)	-1.1 (-2.7, 0.4)	51.5 (7.8)	0.19 (-1.6, 2.0)	0.06 (-1.5, 1.7)
Phase angle °	4.9 (0.6)	5.1 (0.7)	0.14 * (0.0,0.3)	0.07 (-0.0, 0.2)	5.1 (0.5)	0.17 * (0.0,0.3)	0.18 * (0.1, 0.3)

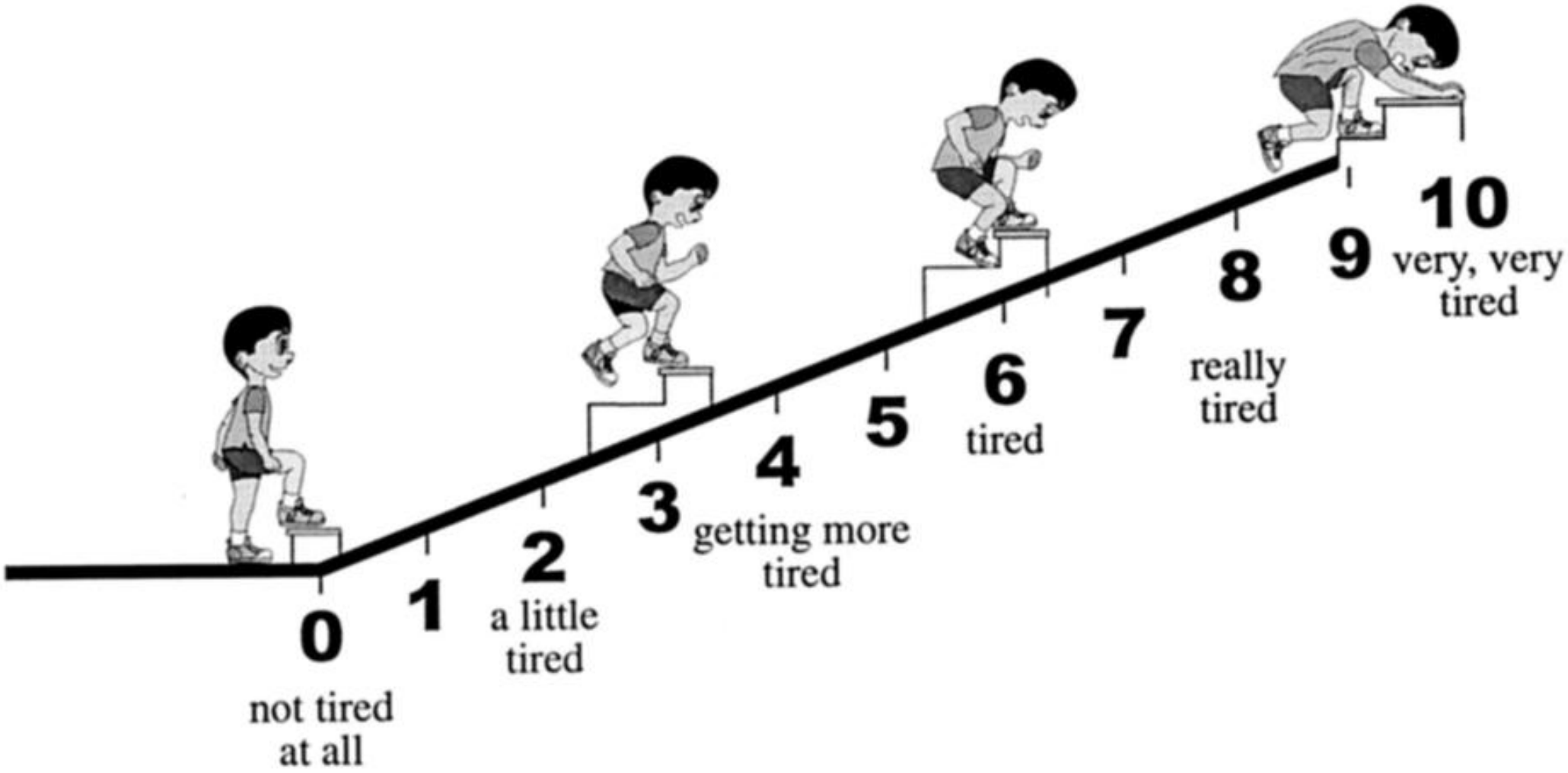
Appendix 17: result of simple and multivariable linear regression analysis for HIV negative and unknown children only for body composition anthropometry outcomes comparing siblings and community controls to cases. Adjusted includes age, sex, puberty and SES

Appendix 18: Body composition outcomes stratified by sex (Adjusted for age, HIV, SES, and puberty status)

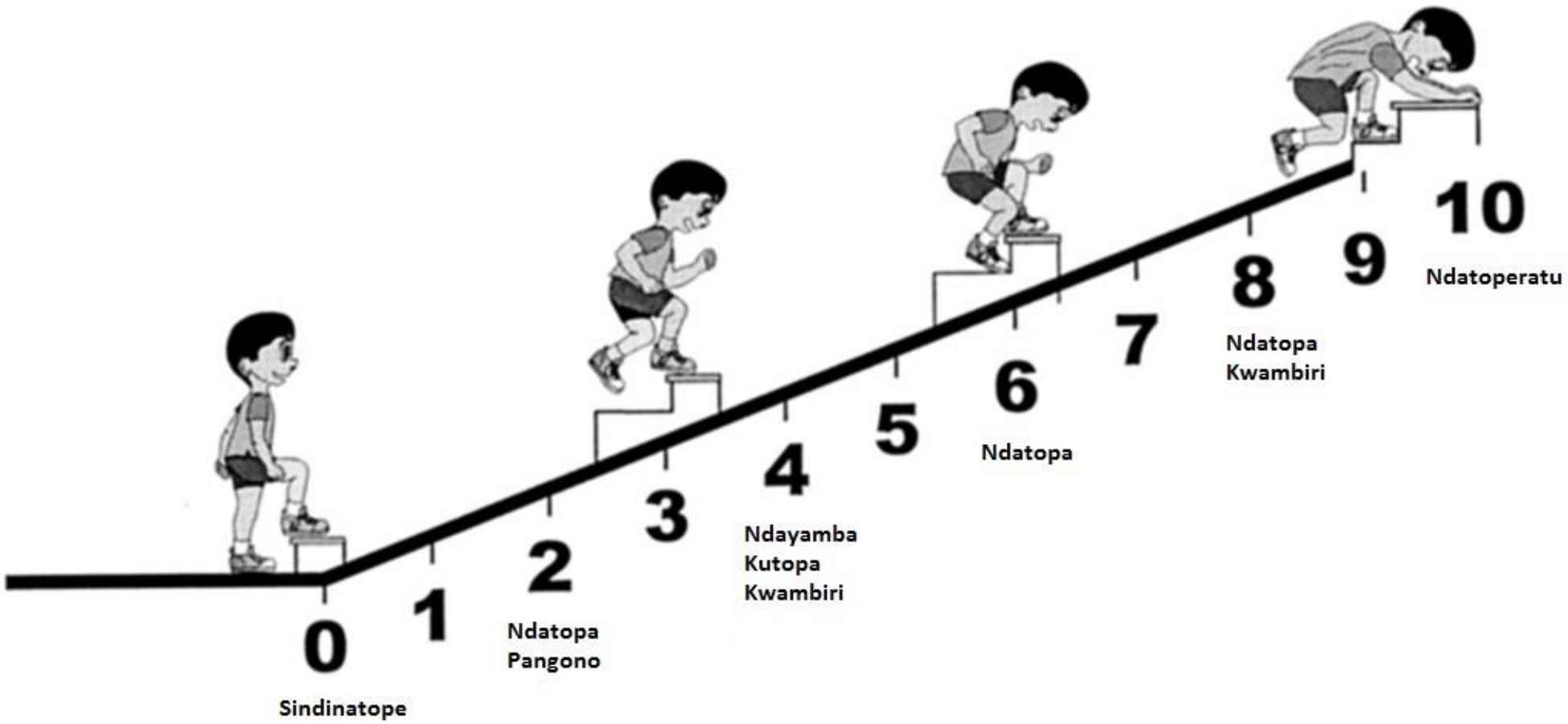
	Cases Girls n=134 Boys n=169		**Siblings** Girls n=106 Boys n=98		**Community** Girls n=56 Boys n=65		
	Mean (SD)	**Mean** (SD)	**Unadjusted difference** (95% CI)	**Adjusted difference** (95% CI)	**Mean** (SD)	**Unadjusted difference** (95% CI)	**Adjusted difference** (95% CI)
Calf circumference							
Girls	23.9 (2.3)	25.3 (4.0)	1.36 * (0.6, 2.1)	0.61 * (0.0, 1.2)	24.6 (2.8)	0.70 (-0.2, 1.6)	0.51 (-0.1, 1.1)
Boys	23.5 (2.3)	24.8 (3.0)	1.30 * (0.7, 1.9)	0.56 * (0.0, 1.1)	24.0 (2.0)	0.50 (-0.1, 1.1)	0.46 (-0.1, 1.0)
Waist circumference							
Girls	55.9 (4.6)	57.6 (7.3)	1.66 * (0.2, 3.1)	0.70 (-0.4, 1.8)	55.4 (4.9)	-0.48 (-2.1, 1.1)	-0.21 (-1.5, 1.1)
Boys	56.6 (3.9)	57.9 (5.4)	1.32 * (0.2, 2.4)	0.38 (-0.6, 1.4)	56.6 (4.1)	-0.01 (-1.2, 1.1)	0.31 (-0.7, 1.3)
Hip circumference							
Girls	62.9 (6.1)	66.7 (12)	3.74 * (1.5, 6.0)	1.79 * (0.2, 3.4)	65.2 (7.9)	2.24 (-0.2, 4.7)	1.77 * (0.0, 3.5)
Boys	61.9 (5.5)	65.0 (8.6)	3.13 * (1.5, 4.8)	1.72 * (0.5, 3.0)	62.4 (5.6)	0.46 (-1.3, 2.2)	1.14 (-0.2, 2.5)
Waist/hip ratio							
Girls	0.89 (0.1)	0.87 (0.1)	-0.02 * (-0.03, -0.0)	-0.01 (-0.0, 0.0)	0.86 (0.1)	-0.03 * (-0.1, -0.0)	-0.02 * (-0.04,-0.0)
Boys	0.92 (0.1)	0.90 (0.1)	-0.02 * (-0.03, -0.0)	-0.01 (-0.0, 0.0)	0.91 (0.1)	-0.01 (-0.02, 0.0)	-0.01 (-0.0, 0.0)
Skinfold ratio							
Girls	1.70 (0.4)	1.75 (0.4)	0.05 (-0.1, 0.2)	0.07 (-0.0, 0.2)	1.70 (0.4)	-0.00 (-0.1, 0.1)	0.04 (-0.1, 0.2)
Boys	1.73 (0.4)	1.76 (0.5)	0.03 (-0.1, 0.1)	-0.00 (-0.1, 0.1)	1.77 (0.4)	0.04 (-0.1, 0.2)	0.04 (-0.1, 0.2)
1/z (lean mass)							
Girls	8.86 (0.9)	8.91 (0.8)	0.05 (-0.2, 0.3)	0.07 (-0.2, 0.3)	8.84 (0.8)	-0.02 (-0.3, 0.2)	-0.04 (-0.3, 0.2)
Boys	9.16 (1.2)	9.51 (1.0)	0.35 * (0.1, 0.6)	0.29 (-0.2, 0.6)	9.37 (1.0)	0.21 (-0.1, 0.5)	0.12 (-0.2, 0.4)
BMI residual (fat mass)							
Girls	-0.06 (1.3)	0.47 (2.2)	0.53 * (0.1, 1.0)	0.30 (-0.1, 0.7)	0.09 (1.9)	0.14 (-0.4, 0.7)	0.26 (-0.2, 0.7)
Boys	-0.22 (1.1)	0.08 (1.1)	0.30 * (0.0, 0.6)	0.10 (-0.2, 0.4)	-0.23 (1.1)	-0.01 (-0.3, 0.3)	0.03 (-0.3, 0.3)
R/h							
Girls	567 (130)	585 (137)	-25.3 (-55, 4.1)	-5.78 (-30, 19)	586 (98)	-24.5 (-57, 8.3)	-20.5 (-48, 6.9)
Boys	610 (98)	548 (119)	-49.8 * (-77, -22)	-21.0 (-46, 4.1)	569 (84)	-28.8 (-58, 0.1)	-26.7 * (-53, -0.3)
Xc/h							
Girls	52.8 (8.9)	51.5 (9.3)	-1.39 (-3.7, 0.9)	0.86 (-1.3, 3.0)	52.5 (7.4)	-0.34 (-2.9, 2.2)	1.26 (-1.2, 3.7)
Boys	51.7 (8.0)	47.8 (9.0)	-3.92 * (-6.0, -1.8)	-1.96 (-4.1, 0.2)	50.6 (8.0)	-1.06 (-3.3, 1.2)	-0.55 (-2.8, 1.7)
Phase angle							
Girls	4.98 (0.7)	5.14 (0.7)	0.16 (-0.0, 0.3)	0.19 * (0.0, 0.4)	5.12 (0.5)	0.15 (-0.0, 0.3)	0.26 * (0.1, 0.5)
Boys	5.01 (0.7)	5.04 (0.7)	0.03 (-0.1, 0.2)	-0.02 (-0.2, 0.1)	5.10 (0.5)	0.09 (-0.1, 0.3)	0.13 (-0.0, 0.3)

Appendix 19: Images of the English and Chichewa versions OMNI and VAS scales

OMNI:



OMNI:

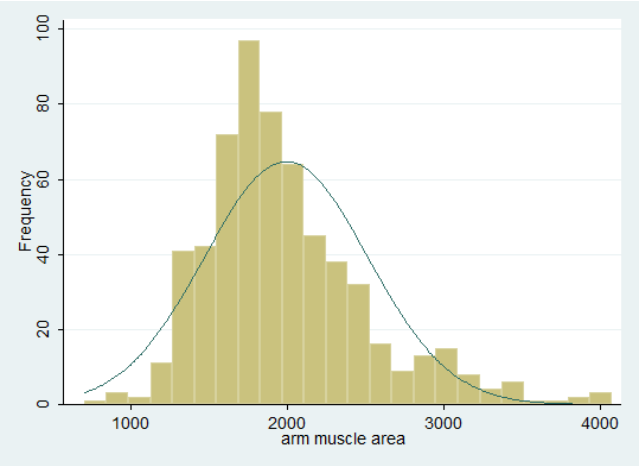

0
Sindinatope
1
2
Ndatopa
Pangono
3
4
Ndayamba
Kutopa
Kwambiri
5
6
Ndatopa
7
8
Ndatopa
Kwambiri
9
10
Ndatoperatu

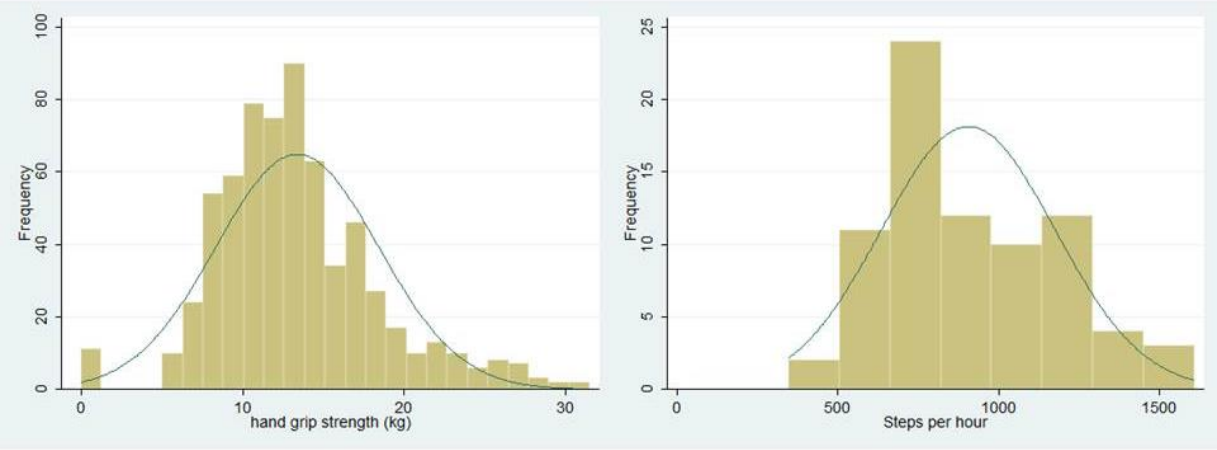
VAS:

Visual Analogue Scale

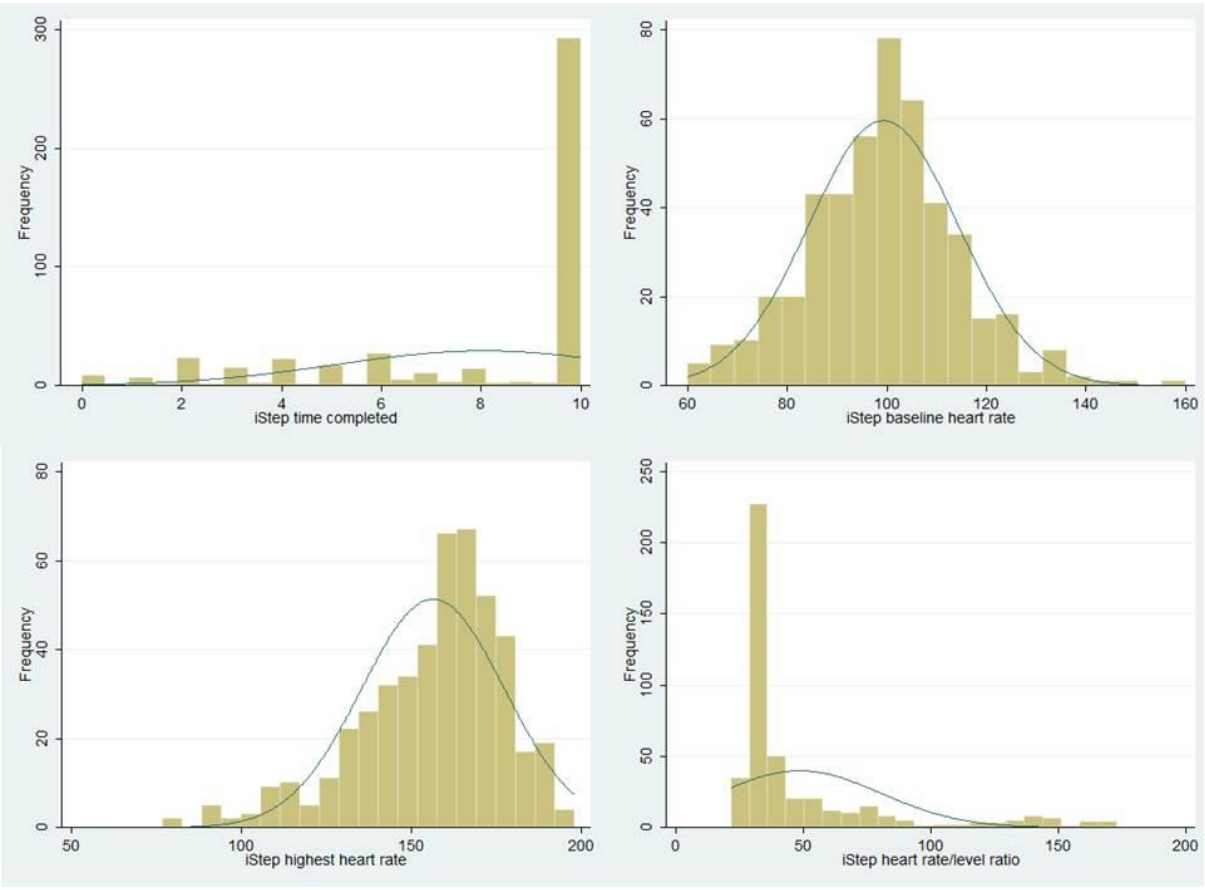
Appendix 20: Histograms of Physical activity outcomes

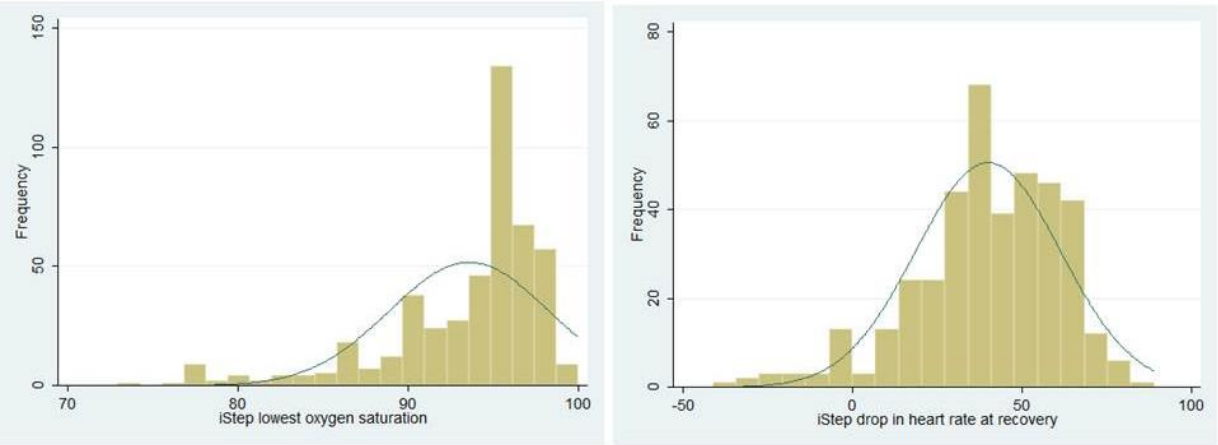
Arm muscle area (AMA), Hand grip strength and Steps per hour





iStep Outcomes





Appendix 21: Table of results comparing physical activity outcomes for HIV negative children only

	HIV- Cases n=210	HIV- Siblings n=206			HIV- Community n=175		
	Mean (SD)	Mean (SD)	Unadjusted difference (95% CI)	Adjusted difference (95% CI)	Mean (SD)	Unadjusted difference (95% CI)	Adjusted difference (95% CI)
Muscle Strength							
Hand grip strength (kg) n= 550	12.3 (3.8)	14.6 (6.8)	2.26 * (1.3, 3.3)	1.03 * (0.3, 1.7)	13.8 (3.9)	1.47 * (0.4, 2.5)	1.66 * (0.9, 2.4)
AMA (cm^2)	1877.4 (440)	2170.1 (728)	292.6 * (176, 410)	152.8 * (63.4,242)	2038.8 (529)	161.3 * (35.1, 288)	165.8 * (68.6, 263)
Physical Activity							
Steps per hour n=63	839.9 (292)	1017.4 (296)	177.5 * (5.5, 350)	202.3 * (16.5,388)	860.6 (213)	20.76 (-143, 184)	60.84 (-122, 234)
Physical Capacity (iStep outcomes)(n=778)							
time completed (minutes)~log	7.64 (3.2)	8.45 (2.8)	0.17~ * (0.0, 0.3)	0.10~ (-0.0, 0.2)	8.39 (2.7)	0.14~ * (0.0, 0.3)	0.14~ * (0.0, 0.3)
Baseline HR	100.3 (14.8)	98.5 (15.7)	-1.81 (-5.3, 1.7)	-0.67 (-4.2, 2.8)	99.2 (14.6)	-1.14 (-4.8, 2.5)	-1.50 (-5.1, 2.1)
Highest HR	154.4 (20.3)	157.7 (20.5)	3.34 (-1.4, 8.1)	1.15 (-3.7, 6.0)	159.0 (19.9)	4.58 (-0.4, 9.5)	4.47 (-0.4, 9.4)
Lowest o2 saturation	93.6 (4.7)	93.8 (5.0)	0.25 (-0.8, 1.3)	0.79 (-0.3, 1.9)	93.7 (3.9)	0.12 (-1.0, 1.2)	0.30 (-0.8, 1,4)
HR at 1 min recovery	112.7 (17.7)	113.3 (19.4)	0.68 (-3.8, 5.1)	1.13 (-3.5, 5.8)	111.2 (18.0)	-1.46 (-6.2, 3.2)	-1.50 (-6.2, 3.1)
Drop in HR at recovery (as % of highest HR)	72.49 (10.3)	70.64 (10.6)	-1.85 (-4.3, 0.6)	-0.78 (-3.4, 1.8)	68.86 (9.9)	-3.62 * (-6.3, -1.0)	-3.69 * (-6.3, -1.1)

Appendix 21: mean (SD) as well we result of simple and multivariable linear regression analysis for comparing physical function outcomes by study group for HIV negative children only. ~ indicates that natural log of the raw value is being used

Appendix 22: Poster exploring relevance of GLI equation for our data

Selecting a spirometry control group for Malawian children

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Introduction:

Lung Function is an important marker of chronic disease in developing countries, but interpreting results can be challenging.

Aim: To determine whether the GLI-Black spirometry reference equation (Quanjer, ERJ 2012) is appropriate for use in Malawian children.

Methods:

- Identical anthropometry and spirometry were undertaken in healthy children participating in:

1) The 'Chronic Disease Outcomes following Severe Acute Malnutrition' (ChroSAM) study (Blantyre, Malawi)
2) The 'Size and Lung Function in Children' (SLIC) study (London, UK)

- Training and quality control conducted by the same physiologist.
- Malawian and UK-Black African (UK BA) spirometry data were expressed as z-scores using the GLI-Black equation.
- Anthropometry z-scores were calculated with the WHO2007 equations.

Results:

Table 1: Comparison of UK Black African and Malawian control children

	UK:Black African	Malawi	Mean Diff (UK-Malawi) (95% CI)
n (% male)	460 (39%)	193 (49%)	
Age (years)	8.7 (1.6)	9.1 (1.5)	-0.5 (-0.7;-2.0)
Height z-score	1.2 (1.1)	-1.2 (1.0)	2.4 (2.2; 2.6)
Weight z-score	1.0 (1.0)	-1.2 (1.3)	2.2 (2.0; 2.4)
BMI z-score	1.0 (1.2)	-0.7 (0.9)	1.7 (1.5; 1.9)
Sit/stand ht (%)	51.8 (1.6)	52.0 (1.6)	-0.2 (-0.5;0.1)
FEV_1 z-score	-0.01 (0.9)	-0.34 (1.0)	0.3 (0.2; 0.5)
FVC z-score	0.24 (1.0)	-0.23 (1.0)	0.5 (0.3; 0.6)
FEV_1/FVC z-score	-0.32 (1.0)	-0.15 (1.0)	-0.3 (-0.4;-0.2)

- The proportion of acceptable results was similar in both groups (71-76%).
- In comparison to UK BA children, Malawian children were:
  - Significantly lighter, shorter and lower BMI
  - FEV_1 and FVC were significantly lower in the Malawian controls
- The spirometric deficits were not as extreme as those observed for height and weight.
- There was no significant difference in sit/stand height ratio (Table 1).

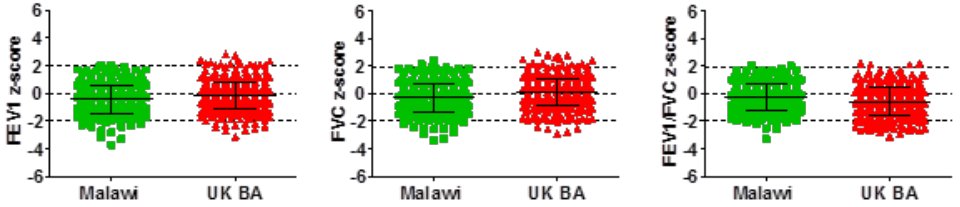

Figure 1: Spirometry results in UK Black African and Malawian control children

Conclusions:

- Despite large height and weight differences between UK-BA and Malawian children, sit/stand height ratio was similar and spirometric differences were small, with no evidence of obstructive lung disease from the FEV_1/FVC ratio suggesting relative preservation of lung and airway size.
- Although the GLI-Black equation fitted the Malawian children reasonably well, interpretation of LF in developing countries with secular changes in somatic growth will be enhanced by recruiting local controls.

Funding support: Wellcome Trust

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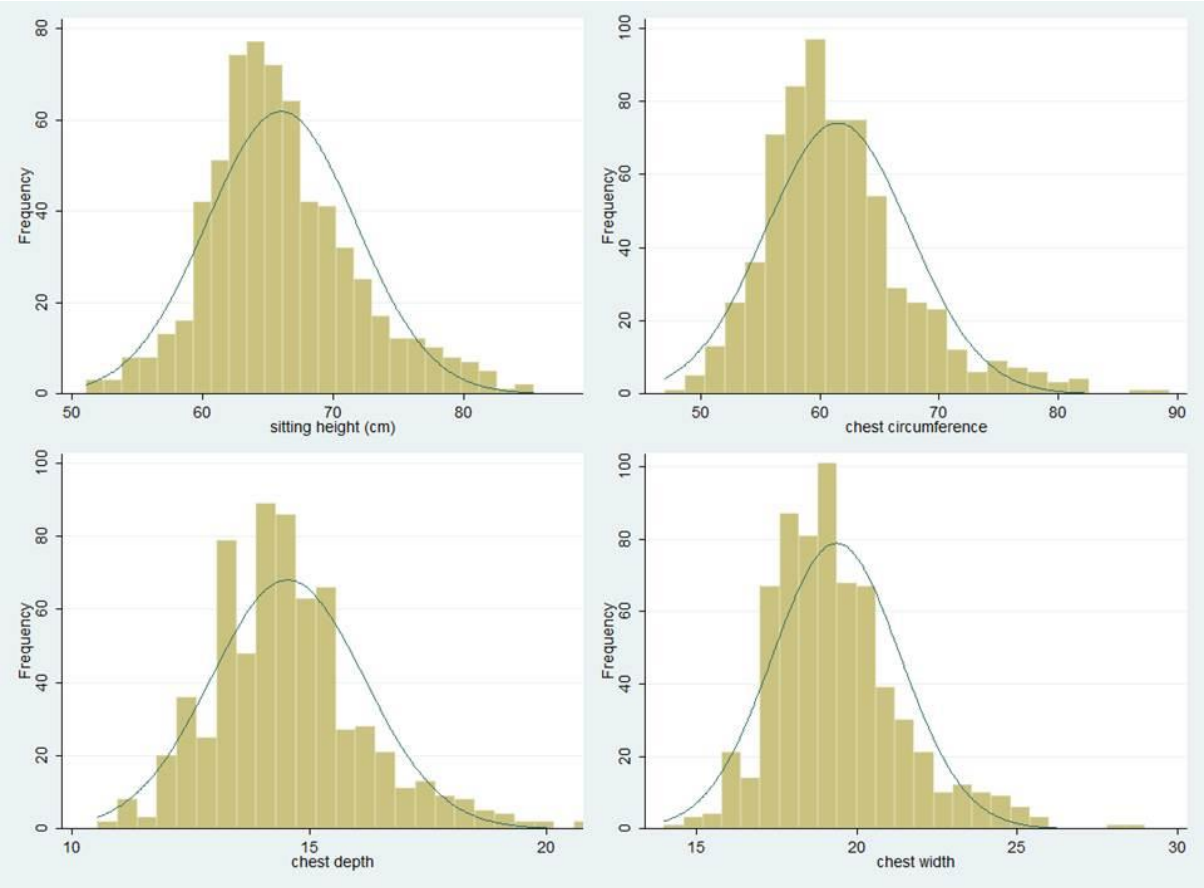
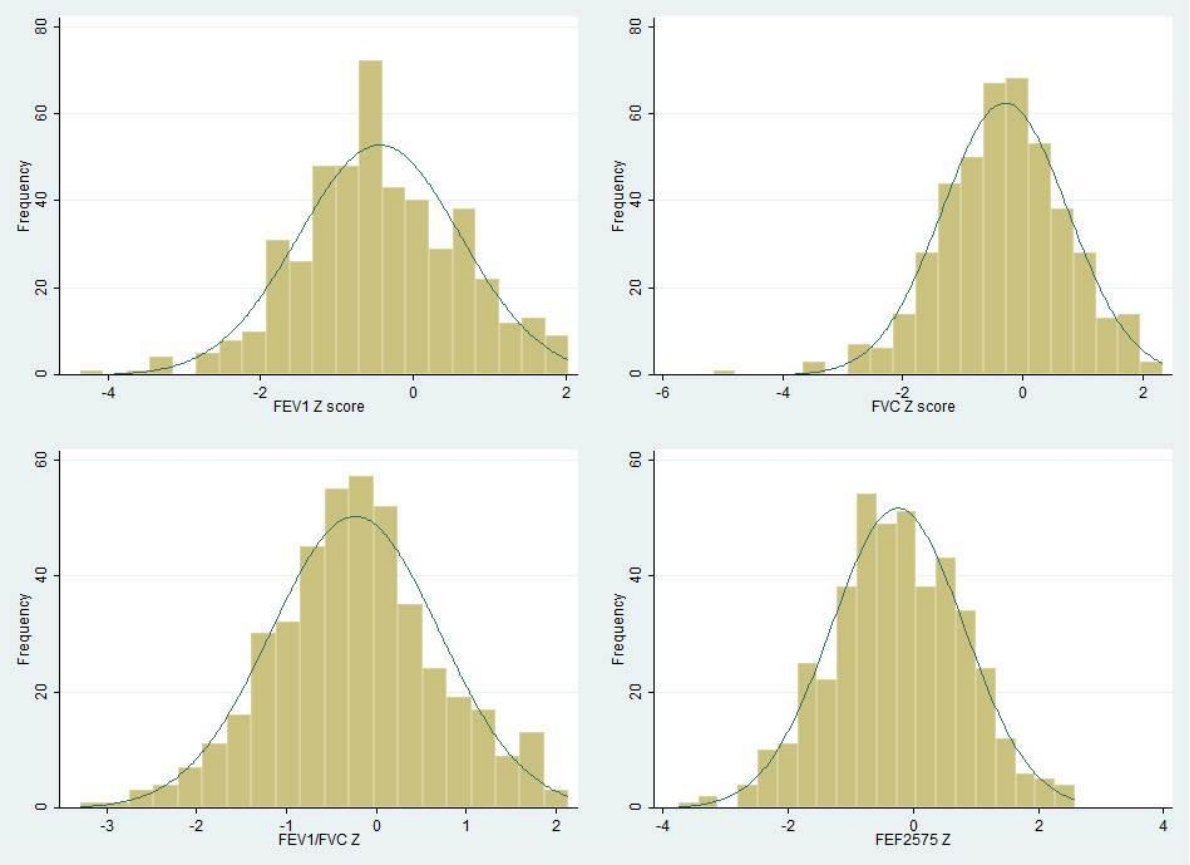
Appendix 23: Histograms of Lung function outcomes

Anthropometry outcomes:



Histograms of sitting height, chest circumference, chest depth and chest width; these outcomes are analysed in Chapter 7: Lung Function

Spirometry Outcomes:



Histograms of spirometry outcomes FEV1, FVC FEV_1/FVC and FEF_{25-75} z scores; these outcomes are analysed in Chapter 7: Lung Function

Appendix 24: Table of results comparing lung function outcomes for HIV negative children only

	HIV- Cases (n=146)	HIV- Sibling (n=136)			HIV- Community (n=114)		
	Mean (SD)	Mean (SD)	Unadjusted difference (95% CI)	Adjusted difference (95% CI)	Mean (SD)	Unadjusted difference (95% CI)	Adjusted difference (95% CI)
FEV_1 (litres)	1.26 (0.3)	1.55 (0.5)	0.29 * (0.2, 0.4)	0.05 (-0.0, 0.1)	1.34 (0.3)	0.08 (-0.0, 0.2)	0.03 (-0.0, 0.1)
FVC (litres)	1.45 (0.3)	1.78 (0.6)	0.32 * (0.2, 0.4)	0.03 (-0.0, 0.10	1.55 (0.4)	0.10 (-0.0, 0.2)	0.05 (-0.0, 0.1)
FEV_1:FVC	0.88 (0.1)	0.89 (0.1)	0.01 (-0.0, 0.0)	0.02 * (0.0, 0.0)	0.87 (0.1)	-0.01 (-0.0, 0.0)	-0.01 (-0.0, 0.0)
FEV_1 Z score	-0.32 (1.0)	-0.51 (1.0)	-0.18 (-0.4, 0.1)	-0.19 (-0.4, 0.1)	-0.31 (1.1)	0.01 (-0.2, 0.3)	-0.02 (-0.3, 0.2)
FVC Z score	-0.19 (0.9)	-0.41 (1.0)	-0.22 (-0.5, 0.0)	-0.26 * (-0.5, -0.0)	-0.13 (1.0)	0.06 (-0.2, 0.3)	0.03 (-0.2, 0.3)
FEV_1:FVC Z score	-0.16 (1.0)	-0.14 (0.9)	0.02 (-0.2, 0.3)	0.10 (-0.1, 0.3)	-0.34 (1.0)	-0.18 (-0.4, 0.1)	-0.18 (-0.4, 0.1)

Appendix 24: Adjusted for SES, sitting height and puberty, raw litres also adjusted for age and sex

Appendix 25: Table of results comparing lung function outcomes stratified by sex

	Cases Girls n=92 Boys n=108		Siblings Girls n=74 Boys n=67		Community Girls n=56 Boys n=65		
	Mean (SD)	**Mean** (SD)	**Unadjusted difference** (95% CI)	**Adjusted difference** (95% CI)	**Mean** (SD)	**Unadjusted difference** (95% CI)	**Adjusted difference** (95% CI)
FEV_1 (litres)							
Girls	1.22 (0.3)	1.52 (0.5)	0.30 * (0.2, 0.4)	0.07 * (0.0, 0.1)	1.34 (0.4)	0.12 (-0.0, 0.2)	0.09 * (0.0, 0.2)
Boys	1.29 (0.3)	1.58 (0.7)	0.30 * (0.2, 0.4)	0.06 (-0.0, 0.1)	1.33 (0.3)	0.04 (-0.1, 0.2)	0.01 (-0.1, 0.1)
FVC (litres)							
Girls	1.41 (0.3)	1.74 (0.5)	0.33 * (0.2, 0.5)	0.06 (-0.0, 0.1)	1.53 (0.4)	0.12 (-0.0, 0.3)	0.09 * (0.0, 0.2)
Boys	1.48 (0.3)	1.82 (0.6)	0.33 * (0.2, 0.5)	0.04 (-0.1, 0.1)	1.56 (0.4)	0.07 (-0.1, 0.2)	0.04 (-0.1, 0.1)
FEV_1:FVC							
Girls	0.88 (0.1)	0.89 (0.1)	0.00 (-0.0, 0.0)	0.01 (-0.0, 0.0)	0.88 (0.1)	-0.00 (-0.0, 0.0)	0.00 (-0.0, 0.0)
Boys	0.87 (0.1)	0.89 (0.1)	0.01 (-0.0, 0.0)	0.03 * (0.0, 0.1)	0.86 (0.1)	-0.01 (-0.0, 0.0)	-0.01 (-0.0, 0.0)
FEV_1 Z score							
Girls	-0.64 (1.1)	-0.66 (0.8)	-0.02 (-0.3, 0.3)	-0.14 (-0.5, 0.2)	-0.40 (1.1)	0.24 (-0.1, 0.6)	0.17 (-0.2, 0.6)
Boys	-0.32 (1.1)	-0.30 (1.1)	0.03 (-0.3, 0.4)	-0.08 (-0.5, 0.3)	-0.29 (1.2)	0.04 (-0.3, 0.4)	-0.15 (-0.5, 0.2)
FVC Z score							
Girls	-0.46 (1.0)	-0.50 (0.9)	-0.03 (-0.3, 0.3)	-0.21 (-0.6, 0.1)	-0.27 (1.0)	0.19 (-0.1, 0.5)	0.10 (-0.3, 0.5)
Boys	-0.20 (1.0)	-0.25 (1.2)	-0.06 (-0.4, 0.3)	-0.22 (-0.6, 0.2)	-0.05 (1.1)	0.14 (-0.2, 0.5)	-0.04 (-0.4, 0.3)
FEV_1:FVC Z score							
Girls	-0.23 (0.9)	-0.25 (0.9)	-0.02 (-0.3, 0.3)	0.04 (-0.3, 0.4)	-0.30 (0.9)	-0.07 (-0.4, 0.2)	-0.04 (-0.4, 0.3)
Boys	-0.20 (0.9)	-0.03 (0.9)	0.17 (-0.1, 0.5)	0.26 (-0.1, 0.6)	-0.44 (1.1)	-0.24 (-0.5, 0.1)	-0.25 (-0.6, 0.1)

Appendix 25: Adjusted for SES, HIV status, sitting height and puberty, raw litres also adjusted for age

Appendix 26: Glossary of Terms

- **BIA:** Bioelectrical Impedance Analysis (BIA) is a technique used for measuring body composition. It uses the principle that electric current flows at different rates through the body depending upon its composition of fat and water. It therefore uses measures of Resistance and Reactance (together known as Impedance) along with height to estimate total body water, fat mass and lean mass.
- **BIVA:** BIA Vector Analysis (BIVA) is a method used for analysing BIA data without the need for population-specific conversions equations. BIVA allows for comparison of lean mass and hydration levels using BIA data. Comparing impedance values directly does not allow for differentiation between high lean mass and high hydration. However BIVA plots the two components of impedance (resistance (R) and reactance (Xc)) as a vector on a graph allowing for differentiation between hydration and lean mass. The arc tangent of the vector graph equates to the phase angle. Long vectors with a small phase angle (increased R with decreased Xc) are associated with malnutrition, whereas short vectors with a small phase angle (decreased R with decreased Xc) are associated with fluid overload.
- **BMI:** Body Mass Index (BMI) is a simple index of weight-for-height that is commonly used to classify underweight, overweight and obesity in older children and adults. It is defined as the weight in kilograms divided by the square of the height in metres (kg/m^2). The WHO defines BMI<18.5 as underweight and BMI $\geq$ 25 as overweight.
- **CMAM:** Community-Based Management of Acute Malnutrition (CMAM) is a methodology for active-case finding and treatment of acute malnutrition in the community. The introduction of Ready-to-Use-Therapeutic Foods (RUTF) enabled the development of CMAM as RUTF can easily be administered at home by care-givers. The majority of acute malnutrition cases found can be treated as outpatient cases using RUTF; only those cases with presence of nutritional oedema, loss of appetite or other concurrent illnesses need to be admitted for inpatient treatment. The CMAM model was developed by Valid International and has been endorsed by WHO and UNICEF.
- **COPD:** Chronic Obstructive Pulmonary Disease is a group of lung diseases characterized by chronic inflammatory obstruction of lung airflow that interferes with normal breathing and is not fully reversible. The more familiar terms 'chronic bronchitis' and 'emphysema' are no longer used, but are now included within the COPD diagnosis.
- **DOHaD:** The developmental origins of health and disease theory (DOHaD) is a multi-disciplinary field of research that examines how environmental factors during the phase of developmental plasticity (i.e. prenatal or early postnatal life) interact with genotypic variation to change the capacity of the organism to cope with is environment in later life, often resulting in adult diseases.
- **FEV_1:** Forced expiratory volume in one second is the volume of air exhaled during the first second of a forced expiratory manoeuvre.
- **FEV_1/FVC:** Forced expiratory volume in one second (FEV_1) expressed as a proportion of the forced vital capacity (FVC) is the fraction of the total that is exhaled in the first second. It is the index of the speed of expiratory airflow. It is calculated by using the largest FEV_1 and the largest FVC, even if they are not from the same curve. A low FEV_1/FVC is associated with airways obstruction
- **FVC:** Forced Vital Capacity is the maximal volume of air which can be exhaled forcefully after maximal inspiration. NOTE: The vital capacity is the amount of air that can be exhaled

by an individual after taking the deepest breath possible, whether or not the air is exhaled forcefully (FVC) or slowly (VC).

- **Impedance:** Impedance (Z) is the degree to which a medium slows or stops an electrical current. When referred to as an outcome from BIA, high impedance indicates low water tissues such as adipose. Muscle has about 75% water, so it has low impedance. It is measured in Ohms.
- **Kwashiorkor:** Kwashiorkor is a form of severe acute malnutrition (SAM) characterised by too much fluid in body tissues which causes swelling under the skin (called oedema). It usually begins in the legs, but can involve the whole body, including the face. An enlarged abdomen, irritability, yellow hair, drowsiness and patches of inflamed or peeling skin may also be present. If the condition is left untreated, it can be fatal. The word derives from the Ghanaian language meaning "weaning sickness" as it often presents in children around the age of weaning. The exact physiological causes behind kwashiorkor are still unknown. Children with kwashiorkor are considered to have "complicated SAM" and must therefore be treated as inpatients in health facilities.
- **Lean mass index (LMI):** Lean mass, usually calculated from BIA readings, can be converted to LMI by diving it by the square of height (lean mass (kg) / height $(m)^2$). Lean mass calculated from BIA is not adjusted for body size, hence LMI provide a discrete measure of *relative* lean mass, expressed in the same kg/m2 units as BMI. When an appropriate conversion equation is not available to convert BIA readings into lean mass (kg), a useful "LMI equivalent" can be calculated simply by dividing 1/Resistance (R).
- **Low Birth Weight (LBW):** LBW is most commonly defined as an infant weighing less than 2500g at birth; this can include infants born early. A more specific term now commonly used is "small for gestational age" which takes preterm births into account.
- **MAM:** Moderate Acute Malnutrition (MAM) is characterised by moderate wasting defined by a weight-for-height indicator between -3 and -2 z-scores of the WHO international growth standard, or by a mid-upper arm circumference (MUAC) between 11.5cm and 12.5cm. If MAM is not treated, it can develop further and become "severe acute malnutrition" (SAM). MAM can be treated with RUTF in the community.
- **Marasmus:** Marasmus is one form of severe acute malnutrition characterised by muscle mass loss. The term marasmus is derived from the Greek word *marasmos*, which means withering or wasting.
- **Non-Communicable Diseases (NCDs):** these are a group of medical conditions or diseases that are non-infectious or non-transmissible. NCDs often refer to chronic diseases which last for long periods of time and progress slowly. The 4 main types of NCDs are cardiovascular diseases (like heart attacks and stroke), cancers, chronic respiratory diseases (such as chronic obstructed pulmonary disease and asthma) and diabetes. Some people might be surprised to learn that NCDs now disproportionately affect low- and middle-income countries where nearly three quarters of NCD deaths – 28 million – occur.
- **Phase angle:** this is a derived measure obtained from the relation between the direct measures of resistance and reactance. It is useful because it does not depend on any conversion equations. It is thought to reflect cell wall health however its exact biological meaning and pathogenic effects are not completely understood.
- **Physical activity:** this is a measure of how physically active someone is during their everyday life; it is technically defined as a body movement that requires energy expenditure however the measure of interest is usually how much it varies in amount, length and/or intensity.

- **Physical capacity:** an individual's physical capacity, also known as exercise capacity, is measured by their ability to endure exercise and/or the maximum workload achieved during the exercise period. Physical capacity provides information on the functioning of several body systems, including circulation, cardiovascular, pulmonary, muscle action and neural function; small deficits in one or some of these areas may only be observed during strenuous effort such as exercise
- **Reactance:** (Xc) this is a component of impedance. It is a measure of the amount of current which a medium can SLOW. For example, cell membranes can store the current for a short period of time, and this slows it down – if it can store, it is called a Capacitor. At low frequencies (50khz), cell membranes are Resistors (current can't pass through) hence reactance only makes up 5% of impedance
- **Resistance:** (R) this is a component of impedance. It is a measure of the amount of current which a medium can STOP. Water has very low resistance hence the less water in the body, the more resistance. Resistance makes up around 95% of impedance
- **RUTF:** Ready-to-Use-Therapeutic Food is a treatment prescribed for malnutrition sufferers. It requires no preparation, no cooking and no addition of water so it is ready-to-use and is fortified with vitamins and minerals; it is usually made of a peanut paste mixed with dry milk products, high in calories to encourage weight gain. It's invention transformed malnutrition treatment as mother's can give RUTF to their children at home without the need for admission to health centres if they have no complications.
- **SAM:** Severe acute malnutrition is defined by a very low weight for height (below -3z scores of the median WHO growth standards); by low MUAC (<11.5cm); by visible severe wasting, or by the presence of nutritional oedema**.** SAM is the most dangerous form of malnutrition; if left untreated, it can result in death. Until 2006, the recommendation was to refer these children to hospital to receive therapeutic diets along with medical care. Treatment recommendations have now changed following the advent of "ready to use therapeutic foods" (RUTF) which allows the management of SAM in the community for those children above the age of 6 months and without medical complications.
- **Skinfold thickness ratio:** skinfold thickness is a measure of subcutaneous fat levels; the ratio is intended to assess relative levels of core to peripheral subcutaneous fat levels. Core skinfold measures include subscapular and suprailiac sites whereas peripheral sites are biceps and triceps. Skinfold thickness ratio is most commonly calculated by adding the subscapular to suprailiac reading and diving this by the tricep reading
- **Spirometry:** (meaning "*the measuring of breath*") is the most common of the pulmonary function tests, measuring lung function. Specifically it measures the amount (volume) and/or speed (flow) of air that can be inhaled and exhaled. It can be used to diagnose asthma, COPD and other conditions that affect breathing.
- **The LMS method:** The LMS method provides a way of obtaining normalized growth centile standards by summarising the changing distribution of a measure (e.g. skinfold thickness) according to a covariate (e.g. age). It assumes that the data can be normalized by using a power transformation, which stretches one tail of the distribution and shrinks the other, removing the skewness. Using a range of "normal" measurements across within an age bracket, the method calculates the optimal power to obtain normality and the trend is summarized by a smooth (L) curve. Trends in the mean (M) and coefficient of variation (S) are also smoothed. The resulting L, M and S curves contain the information to draw a centile curve which is used to generate a reference table with approximate standard errors for the smoothed centiles for each age category. This then allows for comparison of one child's measurements to the group "norm" for that age category,

- **Wasting:** wasting is a form of severe acute malnutrition; it can also be referred to as Marasmus. Wasting is characterised by a loss of muscle mass, usually assessed by anthropometry; weight-for-height and mid-upper arm circumference (MUAC) are the measures recommended by the WHO.